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## **Genome-wide Association Studies Identify Genetic Loci Associated with Albuminuria in Diabetes**

Teumer, Alexander ; Luciani, Alessandro ; Devuyst, Olivier ; et al

**Abstract:** Elevated concentrations of albumin in the urine, albuminuria, are a hallmark of diabetic kidney disease and associate with increased risk for end-stage renal disease and cardiovascular events. To gain insight into the pathophysiological mechanisms underlying albuminuria, we conducted meta-analyses of genome-wide association studies and independent replication in up to 5,825 individuals of European ancestry with diabetes mellitus and up to 46,061 without diabetes, followed by functional studies. Known associations of variants in CUBN, encoding cubilin, with the urinary albumin-to-creatinine ratio (UACR) were confirmed in the overall sample ( $p=2.4 \times 10^{-10}$ ). Gene-by-diabetes interactions were detected and confirmed for variants in HS6ST1 and near RAB38/CTSC. SNPs at these loci demonstrated a genetic effect on UACR in individuals with but not without diabetes. The change in average UACR per minor allele was 21% for HS6ST1 and 13% for RAB38/CTSC ( $p=6.3 \times 10^{-7}$  and  $5.8 \times 10^{-7}$ , respectively). Experiments using streptozotocin-treated diabetic Rab38 knockout and control rats showed higher urinary albumin concentrations and reduced amounts of megalin and cubilin at the proximal tubule cell surface in Rab38 knockout vs. control rats. Relative expression of RAB38 was higher in tubuli of patients with diabetic kidney disease compared to controls. The loci identified here confirm known and highlight novel pathways influencing albuminuria.

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# Genome-wide Association Studies Identify Genetic Loci Associated with

## Albuminuria in Diabetes

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## Abstract

Elevated concentrations of albumin in the urine, albuminuria, are a hallmark of diabetic kidney disease and associate with increased risk for end-stage renal disease and cardiovascular events. To gain insight into the pathophysiological mechanisms underlying albuminuria, we conducted meta-analyses of genome-wide association studies and independent replication in up to 5,825 individuals of European ancestry with diabetes mellitus and up to 46,061 without diabetes, followed by functional studies. Known associations of variants in *CUBN*, encoding cubilin, with the urinary albumin-to-creatinine ratio (UACR) were confirmed in the overall sample ( $p=2.4 \times 10^{-10}$ ). Gene-by-diabetes interactions were detected and confirmed for variants in *HS6ST1* and near *RAB38/CTSC*. SNPs at these loci demonstrated a genetic effect on UACR in individuals with but not without diabetes. The change in average UACR per minor allele was 21% for *HS6ST1* and 13% for *RAB38/CTSC* ( $p=6.3 \times 10^{-7}$  and  $5.8 \times 10^{-7}$ , respectively). Experiments using streptozotocin-treated diabetic *Rab38* knockout and control rats showed higher urinary albumin concentrations and reduced amounts of megalin and cubilin at the proximal tubule cell surface in *Rab38* knockout vs. control rats. Relative expression of *RAB38* was higher in tubuli of patients with diabetic kidney disease compared to controls. The loci identified here confirm known and highlight novel pathways influencing albuminuria.



## Introduction

Urinary albumin and serum creatinine are two biomarkers recommended for routine assessment of chronic kidney disease (CKD).(1) Even at physiological rates of glomerular filtration, small elevations in urinary albumin concentrations are associated with an increased risk for CKD progression, end-stage renal disease (ESRD), cardiovascular events and both cardiovascular and all-cause mortality.(2–4) Patients with diabetes mellitus are at particularly high-risk for CKD and its sequelae: the prevalence of CKD among individuals with diabetes is >40% compared to about 10% in the general U.S. adult population,(5) and the presence of CKD is an important contributor to the excess mortality in diabetes.(6) The appearance of significant amounts of albumin in the urine (albuminuria) is a hallmark of diabetic kidney disease (DKD), the incidence of which continues to rise along with type 2 diabetes worldwide.(7) Even in treated individuals, residual diabetes-related microvascular risk represents an important challenge,(8) and DKD remains the leading cause of ESRD. No new effective treatments for DKD have been approved in more than two decades,(9) highlighting the importance to better understand its underlying mechanisms.

Using genome-wide association study (GWAS) meta-analysis in general population cohorts, we previously identified a missense single nucleotide polymorphism (SNP) in the gene encoding cubilin (*CUBN*) in association with the urinary albumin-to-creatinine ratio (UACR).(10) *CUBN* is currently the only genome-wide significant locus for UACR. However, this variant explains only a small fraction of the previously reported heritability of albuminuria ranging from 0.2-0.46 in the general population and those with diabetes,(11–13) suggesting that additional genetic variants remain to be found. Here we report the results of a GWAS meta-analysis of albuminuria traits in the general population performed in almost twice the sample size of our

previous study,(10) with a special focus on those with diabetes, replication in additional independent individuals, and follow-up investigations in human tissues and a genetically modified animal model of diabetes mellitus.

**Research Design and Methods**

*Study Populations*

Our study was based on 30 discovery and replication studies mostly from the general population, with the exception of ADVANCE and GENDIAN that enrolled exclusively individuals with type 2 diabetes, totaling 67,452 participants of European ancestry across the different analyses (up to 7,787 with diabetes in discovery and replication). The study characteristics, including the distribution of albuminuria and diabetes, are shown in **Supplementary Table 1**. Study protocols were approved by each local Institutional Review Board or Ethics Committee, and all human participants gave written informed consent.

*Phenotype Definitions and Analytical Strategy*

The measurement of urinary albumin and creatinine in each study is reported in **Supplementary Table 2**. Urinary albumin values below the detection limit of the used assays were set to the lower limit of detection. Rather than using urinary albumin, the urinary albumin-to-creatinine ratio (UACR) was calculated as urinary albumin/urinary creatinine (mg/g) to account for differences in urine concentration. Microalbuminuria (MA) was defined as UACR >25 mg/g in women and >17 mg/g in men.(10) Diabetes was defined as fasting glucose  $\geq$ 126 mg/dl, non-fasting glucose  $\geq$ 200 mg/dl or treatment for diabetes, or – if this information was not available - based on self-report. Across studies, we evaluated two traits, UACR and MA, and performed

four GWAS meta-analyses: MA and UACR in the overall sample, as well as UACR – a continuous trait with higher statistical power - separately among those with and without diabetes. Diabetes-stratified genome-wide association analyses of MA were not performed due to limited sample size. Detailed information on each study's design, genotyping, imputation and data management is provided in **Supplementary Tables 2 and 3**.

#### *Discovery Meta-Analysis, Replication and Power*

Stringent quality control of the genetic data was performed at the individual study level and again at the meta-analysis level using state-of-the-art methods. Missing genotypes were imputed using the HapMap reference panels in 19 studies and the 1000 Genomes reference panels in two studies. Details of genotyping, imputation software, reference panels, and quality filters in each study are reported in **Supplementary Table 3**.

All studies performed GWAS following a standardized analysis protocol. In each study, the natural logarithm of UACR was taken. Subsequently, sex-specific residuals were obtained from linear regression models of  $\ln(\text{UACR})$  on age and study-specific covariates, including study center and genetic principal components to adjust for possible population stratification if applicable. The continuous sex-specific residuals were then combined and used as the dependent variable that was regressed on imputed allelic dosages for each SNP in the GWAS.

Prior to meta-analyses, all study-specific GWAS summary files underwent quality control using GWAtoolbox.<sup>(14)</sup> Genomic-control (GC)<sup>(15)</sup> correction was applied when the GC factor was  $>1$ . Inverse-variance weighted fixed-effects meta-analyses were then conducted using METAL.<sup>(16)</sup> The  $I^2$  statistic was used to evaluate between-study heterogeneity.<sup>(17)</sup> All meta-analyses were carried out in duplicate by two independent researchers.

After meta-analysis, SNPs with average minor allele frequency (MAF)  $<0.01$  were excluded, and another GC correction was applied. There were 2,191,945 SNPs with average MAF  $>0.05$  and present in  $>50\%$  of the studies, which were then clustered based on correlation (linkage disequilibrium pruning using  $r^2 \leq 0.2$ ) with the respective index SNP (the SNP with the lowest p-value) within windows of  $\pm 1$  MB to identify independent SNPs with suggestive association ( $p < 10^{-5}$ ) in one or more of the four analyses.

Replication testing was then carried out for signals that were either genome-wide significant ( $p < 5 \times 10^{-8}$ ) in any analysis, or showed suggestive association among those with diabetes, motivated by the clinical importance of DKD and the stronger association of the known and validated *CUBN* variant on UACR among those with diabetes.(18) Replication was defined as a one-sided p-value  $<0.05$  in the meta-analysis of independent replication studies. Of the nine studies that contributed to replication, five studies used imputed dosage, and four studies performed replication genotyping of the index SNPs. A meta-analysis of the replication results was performed. Subsequently, the double GC-corrected results from the discovery meta-analysis and the results of the nine replication studies were meta-analyzed to obtain the overall statistical significance. Unless stated otherwise, all reported p-values are two-sided.

Assuming that associated SNPs explain a respective 0.6% and 0.5% of the UACR variance in diabetes (Table 1), there was 95% and 91% power, respectively, to replicate the seven suggestive loci from the discovery stage in an additional 1,800 samples with a 1-sided p-value  $<0.05$ .

#### *Additional Analyses to Characterize Novel Loci*

Replicated SNPs were further evaluated even in the absence of genome-wide significance because, in addition to the significant replication p-value, the low heterogeneity across cohorts and the biological plausibility of the *RAB38* locus further increased confidence in the findings. The SNPs were evaluated in the DCCT/EDIC study for association with a primary clinical endpoint defined as time from DCCT baseline until time to persistent microalbuminuria or a secondary endpoint of time to incident albumin excretion rate >300 mg /24h or end-stage renal disease.(10) Time to outcome development or censoring was determined as the number of visit years from DCCT baseline up to and including the 12th year of EDIC follow-up. Subjects with persistent microalbuminuria at DCCT baseline and DCCT year 1 were excluded from analyses of that outcome.

Epigenomic map analyses were performed as described previously(19) using data from human kidney and kidney proximal tubule epithelial cells that can be accessed at Gene Expression Omnibus (GSE49637).

Genetic associations with additional renal function traits, estimated glomerular filtration rate (eGFR) and CKD, were evaluated based on results from GWAS meta-analysis of the corresponding traits within the CKDGen Consortium (personal communication).

#### *Gene Expression Analyses in Human Tissues*

Quantification of transcript abundance in micro-dissected fractions of human glomeruli and tubuli from surgical nephrectomies, living allograft donors and portions of diagnostic kidney biopsies(20) was carried out using RNA-seq. Tissue from different renal compartments was separated using micro-dissection, homogenized and stored at -80°C. Total RNA of human proximal tubule fractions (n=256) and glomerular cells (n=48) were isolated using RNeasy Mini

Kit (Qiagen) according to manufacturer's instructions. RNA quality was assessed with the Agilent Bioanalyzer 2100, and RNA preparations exhibiting RIN scores >7 were used for cDNA synthesis. (library preparation at DNA Sequencing Core at UT Southwestern Medical Center). In short, 1 ug total RNA was used to isolate poly A purified mRNA using the Illumina TruSeq RNA Preparation Kit. Single-end 100bp sequencing was carried out, and the annotated RNA counts (fastq) were calculated by Illumina's CASAVA 1.8.2. Reads were mapped to the reference genome (NCBI build 37, hg19) using Spliced Transcripts Alignment to a Reference (STAR). Reads per kilobase of transcript per million mapped (RPKM) for *HS6ST1* and *RAB38* were compared between glomerular and tubular fractions using a two-sided t-test.

Comparison of candidate gene expression between cases with biopsy-proven DKD and healthy controls was based on publicly available micro-array data from human micro-dissected glomeruli and tubuli (Gene Expression Omnibus (GSE 30122)).(20) Raw data were analyzed using the R package 'Affy' Version 1.44.0, expression levels were normalized using Robust Multi-array Average (RMA). Transcript abundance between patients and controls was compared using two-sided t-tests; statistical significance was defined as  $p < 8.3 \times 10^{-3}$  (alpha of 0.05 corrected for six comparisons).

### *Studies of Rab38 in Rats*

To better understand the association of *RAB38* with albuminuria in diabetes, we studied genetically modified rat models of diabetes. Eight *Rab38* knockout (KO) rats on a Fawn-hooded hypertensive (FHH) background, seven rats transgenic for the wild-type Brown Norway rat *Rab38* allele, and seven congenic rats were generated and raised as described previously.(21–23) *Rab38* KO rats did not express the protein.(22) These references also describe the recording

of blood pressure and the measurement of glucose and albuminuria. Diabetes was induced by treating 9-week-old male rats with streptozotocin (STZ, Sigma-Aldrich, St. Louis, MO, 50mg/kg i.p.).

Paraffin blocks of rat kidney samples were sectioned (thickness 6µm) with a Leica RM2255 rotary microtome (Thermo-Fisher Scientific, Waltham, MA) on Superfrost Plus glass slides (12-550-15, Thermo-Fisher Scientific, Waltham, MA). Before staining, slides were deparaffinized in changes of CitriSolv (22-143-975, Thermo-Fisher Scientific, Waltham, MA) and 70% isopropanol. Antigen retrieval was accomplished by incubating in sodium citrate buffer (1.8% 0.1M citric acid, 8.2% 0.1M sodium citrate, in distilled water, pH 6.0) in a rice cooker for 30 minutes. Slides were blocked with PBS blocking buffer (1% BSA, 0.2% non-fat dry milk in PBS) for 30 minutes and stained with primary antibodies specific for megalin or cubilin diluted in blocking buffer overnight at 4°C. Sheep anti-megalyn and rabbit anti-cubilin were kindly provided by Dr. P Verroust, INSERM, Paris, France. After two washes in 0.1% Tween 20 (v/v in PBS), slides were incubated with corresponding fluorophore-conjugated secondary antibodies (Invitrogen) diluted in blocking buffer at room temperature for 1 hour and counterstained with 10 µM Hoechst 33342 (Molecular Probes-Invitrogen, H1399). Slides were subsequently mounted in Prolong Gold Anti-fade reagent (Invitrogen), acquired on Leica SP5 confocal laser scanning microscope (Center for Microscopy and Image Analysis, University of Zurich) equipped with a Leica APO 63x NA 1.4 oil immersion objective.

All experiments were performed in compliance with National Institutes of Health Guide for Care and Use of Laboratory Animals, and all used protocols were approved by the local Institutional Animal Care and Use Committee.

## Results

### *Discovery of Genomic Loci Associated with Albuminuria Traits*

The discovery GWAS meta-analyses for the four traits included up to 20 studies and up to 54,450 individuals per trait. The median UACR in the 20 individual studies that contributed to the UACR meta-analysis ranged from 2.5 to 15.6 mg/g. Across all studies, the mean proportion of women was 53%, and the median of average age was 57 years. The prevalence of diabetes in the population-based studies ranged from 1 to 14% (**Supplementary Table 1**).

There was no evidence of systematic biases influencing the genome-wide association results as indicated by low genomic control parameters (**Supplementary Fig. 1**). Only SNPs in the previously identified *CUBN* locus showed genome-wide significant association with both UACR ( $p=2.4 \times 10^{-10}$ , **Supplementary Table 4**, **Supplementary Fig. 2**) and MA ( $p=1.3 \times 10^{-10}$ , **Supplementary Table 5**, **Supplementary Fig. 2**). The effect of the minor C allele of the index SNP rs10795433 on logarithmic UACR values was four-fold larger among 5,825 individuals with diabetes ( $0.19 \log(\text{mg/g})$ ,  $p=2.0 \times 10^{-5}$ ) compared to 46,061 individuals without diabetes ( $0.045 \log(\text{mg/g})$ ,  $p=6.1 \times 10^{-6}$ ,  $p\text{-value for difference } 6.2 \times 10^{-3}$ ). This corresponds, for each additional C allele, to a 5% higher geometric mean of UACR ( $\exp(0.045)$ ) in non-diabetics compared to 21% higher average UACR in diabetics ( $\exp(0.19)$ ).

Suggestive associations were identified for all four analyses (**Supplementary Tables 4–7**, regional association plots in **Supplementary Fig. 3**). Among the clinically important group of individuals with diabetes, seven genomic loci contained one or more SNPs showing suggestive association with UACR. These were exclusively identified in the meta-analysis of individuals with diabetes and mapped into or near *HS6ST1*, *CNTN4*, *KBTBD8*, *TFAP2B/PKHD1*, *CHN2*, *WDR11/FGFR2*, and *RAB38/CTSC* (**Supplementary Table 7**). Following our analytical strategy, we



selected the index SNP in each of these seven regions for follow up among up to 1,962 independent individuals with diabetes.

The Supplementary PDF document contains the QQ and Manhattan plots of all GWAS meta-analyses, the regional association plots, tables with cohort descriptions and association results of SNPs at  $p < 10^{-5}$ .

#### *Replication Analyses Implicate RAB38/CTSC and HS6ST1 as Novel Loci for UACR in Diabetes*

The replication analyses included 9 studies and up to 1,962 individuals with diabetes. The median UACR across replication studies ranged from 3.8 to 14.5 mg/g. The mean proportion of women was 49%, and the median of average age was 55 years. For the seven SNPs tested for replication (**Supplementary Table 8**), we assessed whether the one-sided p-value was  $< 0.05$  in the combined replication studies (see Methods). This was the case for two SNPs: intergenic rs649529 upstream of *RAB38*/downstream of *CTSC* on chromosome 11q14 (**Fig. 1a**) and the intronic variant rs13427836 in *HS6ST1* on chromosome 2q21 (**Fig. 1b**). As illustrated in **Fig. 1c**, each additional copy of the minor T allele of rs649529 at *RAB38/CTSC* was consistently associated with lower UACR among the 5,825 individuals in the discovery and 1,962 in the replication cohorts (combined  $p = 5.8 \times 10^{-7}$ , **Table 1**), with no evidence of heterogeneity across cohorts ( $I^2 = 0\%$ ). This effect corresponded to 13% lower geometric mean of UACR per copy of the T allele. Similarly, rs13427836 in *HS6ST1* showed consistent effects across cohorts (combined  $p = 6.3 \times 10^{-7}$ , **Table 1**), with each copy of the T allele associated with approximately 21% higher mean UACR but moderate heterogeneity ( $I^2 = 29.9\%$ , **Fig. 1d**).

The association of both rs649529 near *RAB38/CTSC* and rs13427836 in *HS6ST1* was not found in individuals without diabetes ( $p = 1.0$  and  $p = 0.76$ , respectively, **Table 2**). Differences in

the association with UACR among those with and without diabetes were significant (t-test for difference  $p=6.9 \times 10^{-6}$  for rs649529 and  $p=1.7 \times 10^{-5}$  for rs13427836). Effects for the index variant in *CUBN* are provided for comparison. We also evaluated the association of the replicated SNPs with MA in the setting of diabetes. Information was obtained from a subset of studies with sufficiently high numbers of individuals with diabetes and MA ( $n=2,552$ ; ARIC, CHS, COLAUS, EPIC, FHS, KORAF3, KORAF4, and SHIP). Across cohorts, the odds ratio (OR) for MA for each copy of the minor allele was 0.84 for rs649529 near *RAB38* ( $p=0.019$ ) and 1.39 for rs13427836 in *HS6ST1* ( $p=7.8 \times 10^{-4}$ ), consistent with the direction of the SNP effects on UACR.

#### *Characterization of Genetic Effects by Markers of Kidney Function and Diabetes*

Next we investigated whether the gene-by-environment interaction was also observed for the eGFR, another measure of kidney function, and/or diabetes or glycemic traits. There were no statistically significant associations between rs649529, rs13427836, rs10795433 and eGFR in those with diabetes or without diabetes (**Table 2**), nor any associations with CKD. There were also no statistically significant associations of these variants with type 2 diabetes, fasting blood glucose, or plasma hemoglobin A1c concentrations (**Table 2**), indicating that the observed associations pertain to albuminuria in the setting of diabetes rather than to diabetes or impaired glucose metabolism *per se*. A comprehensive search in the NHGRI GWAS Catalog(24) did not reveal any significant associations between the two validated SNPs or their proxies with other diseases or traits.

#### *Variant Evaluation*

Using publicly available data of genetic effects on gene expression,(25) we found an association

in *cis* between rs649529 and transcript levels of both *RAB38* ( $p=5.4 \times 10^{-6}$ ) and the neighboring *CTSC* ( $p=7.6 \times 10^{-7}$ ), consistent with a regulatory effect of this variant in whole blood. Corresponding data for kidney-specific tissues are currently not available, but we used epigenetic maps generated from human adult kidney tissue(19) (see Methods) to further examine the regulatory potential of index SNPs. The intronic index SNP in *HS6ST1* and several proxies mapped into enhancer regions. Similarly, the *CUBN* index variant rs10795433 mapped into an intronic enhancer region. The region in which the index variant at *RAB38/CTSC* is located was annotated as not mapped/repressed in these cells preventing further examination. All proxies in strong LD with these three index SNPs ( $r^2 > 0.6$ , 1000G v5 reference panel)(26) were intronic (*CUBN* and *HS6ST1*) or intergenic (*RAB38/CTSC*).

#### *Clinical Characterization Including Gene Expression of Replicated Loci*

In order to evaluate target tissues within the kidney, we characterized the identified loci using tissue-specific gene expression data. Clinical characterization was conducted using data from patients with DKD and healthy controls(27) and a prospective study of individuals with type 1 diabetes.(28)

We utilized publicly available data(27) to compare relative expression of *RAB38*, *CTSC* and *HS6ST1* between patients with biopsy-confirmed DKD and healthy controls (see Methods). After multiple testing correction, only *RAB38* expression levels were significantly different, with higher expression in tubuli of DKD patients compared to controls ( $p=1.3 \times 10^{-4}$ , **Fig. 2a**). We also used RNA-seq data from micro-dissected human kidney samples to quantify *RAB38* and *HS6ST1* expression in human glomeruli and tubuli. *HS6ST1* showed higher expression levels than *RAB38*, and both genes showed higher expression in tubuli than in glomeruli (**Fig. 2b**). The difference

between tubular and glomerular expression was more pronounced for *RAB38* ( $p=1.1 \times 10^{-8}$ ) than for *HS6ST1* ( $p=0.015$ ).

To investigate whether the effect of the replicated SNPs extended to kidney disease progression in the setting of type 1 diabetes, the SNPs were tested for association with incident MA (268 cases, primary endpoint) and a combined endpoint of time to macroalbuminuria or ESRD (133 cases, secondary endpoint) among up to 1,304 participants with type 1 diabetes in the DCCT/EDIC Study.(28) Neither SNP showed significant association (**Supplementary Table 9**).

#### *Diabetic Rab38 Knock-out Rats Show Increased Urinary Albumin Excretion*

We aimed to further substantiate our findings by obtaining experimental support. We focused on the examination of *RAB38* because it was the gene implicated by higher gene expression in tubuli of DKD patients compared to controls, and because previous studies of *Rab38* KO and transgenic rats have confirmed its role in albuminuria in FHH rats and highlighted a role in tubular albumin reuptake.(21,22) We thus examined these animals in the setting of diabetes as outlined in **Fig. 3a**. Injection of streptozotocin (STZ) in 9-week-old rats successfully induced diabetes in all strains (**Fig. 3b**). Blood glucose rose from normal values before injection of STZ (congenic  $205 \pm 3$  mg/dL, transgenic  $227 \pm 11$  mg/dL, KO  $198 \pm 7$  mg/dL) to high values that indicate severe hyperglycemia one week after STZ (congenic  $422 \pm 35$  mg/dL, transgenic  $406 \pm 27$  mg/dL, KO  $420 \pm 21$  mg/dL). At age 11, 12, and 13 weeks, blood glucose levels remained high and showed no significant differences between strains (**Fig. 3b**). There were no significant differences in mean arterial blood pressure between congenic, transgenic, and KO animals freely moving around the cage; all animal strains showed a tendency towards decreased blood pressure 3-4 weeks after injection of STZ (**Fig. 3c**).

As illustrated in **Fig. 3d**, *Rab38* KO animals showed a progressive increase in urinary albumin excretion that became statistically significant two weeks after injection of STZ. At 4 weeks post injection, *Rab38* KO animals had an albumin excretion of  $79 \pm 14$  mg/day, whereas albumin excretion was only  $28 \pm 8$  mg/day in transgenic ( $p < 0.01$ ) and  $41 \pm 13$  mg/day in congenic animals ( $p < 0.01$ ). These data indicate that diabetic rats without *Rab38* are more susceptible to the development of albuminuria than congenic and transgenic animals with functional *Rab38* despite a similar degree of hyperglycemia in all animals. Kidney sections obtained from a subset of animals showed a higher average glomerulosclerosis score ( $2.9 \pm 0.3$ ) compared to congenic ( $2.2 \pm 0.1$ ) and transgenic ( $2.2 \pm 0.1$ ) rats ( $p < 0.05$ , **Supplementary Fig. 4**), but differences were subtler than the ones observed for urinary albumin excretion.

To further clarify how loss of *Rab38* may lead to albuminuria, we performed immunohistochemistry staining of megalin and cubilin, known to mediate albumin re-uptake in the proximal tubulus, in kidney sections of all three animal strains. There was a marked reduction of both cubilin and megalin at the luminal membrane of proximal tubular cells in *Rab38* KO rats compared to congenic and transgenic control animals (**Fig. 3e**), consistent with a role of *Rab38* in regulating the abundance of cubilin and megalin at the cell surface. In contrast, there was no significant difference in the number of structures positive for the lysosomal marker LAMP1 among the three strains.

## Discussion

In this GWAS discovery meta-analysis of 2,191,945 SNPs in up to 54,450 participants of 20 studies, we replicated the association of the previously identified *CUBN* locus and UACR as well as MA at genome-wide significance and identified several suggestive signals among individuals

with diabetes. Two of these loci, *RAB38/CTSC* and *HS6ST1*, showed evidence of independent replication and *RAB38* was further supported by functional studies in a rat model. Our findings point to mechanisms in renal handling of albumin that associate with albuminuria in humans in the setting of diabetes. They thus represent examples of gene-by-diabetes interactions resulting in a complex trait that manifests when both environmental exposure and genetic susceptibility variants occur together.(29)

Not all individuals with diabetes develop DKD, suggesting that neither the presence of hyperglycemia nor genetic variants alone are sufficient to elicit the renal damage that typically manifests itself as albuminuria in diabetes. Our observations therefore raise the question of how the diabetic environment may result in the manifestation of genetic effects on albuminuria. The lack of association between both genetic variants and type 2 diabetes or specific glycemic measures in humans indicates that their effects occur without influencing diabetes *per se*. This notion is further substantiated by the fact that diabetic *Rab38* KO rats showed higher urinary albumin concentrations compared to controls despite the presence of similar blood glucose concentrations.

A difference between our observations in humans and rats is that the effect of genetic variation near *RAB38* on albuminuria was only found in humans with but not without diabetes, whereas *Rab38* KO rats without diabetes also progress to albuminuria.(22) A potential explanation is that KO rats represent a null mutation, allowing for the genetic component to take full effect without needing further aggravation by environmental factors. Conversely, many human susceptibility variants of complex traits do not result in a complete loss of function but instead are of regulatory nature. The effect of such variants may become apparent only upon an

environmental challenge, such that genetically determined alterations in renal albumin handling could manifest themselves in the setting of hyperglycemia and/or diabetes due to a number of mechanisms that secondarily impact albumin reabsorption, including an increased load of filtered albumin due to hyperfiltration or impairment of the glomerular filter. Along these lines, our observation of significantly higher *RAB38* transcript abundance in tubuli of DKD patients than controls may indicate an adaption of the tubular machinery for albumin reabsorption in this setting. Moreover, genetics effects of the index SNP at the *CUBN* locus on albuminuria were four times as large in individuals with compared to those without diabetes, supporting alterations of tubular albumin handling in the setting of diabetes.

*RAB38* encodes a member of the small Rab GTPase protein family that regulate intracellular vesicle trafficking between organelles and are important in exo-, endo- and transcytosis.(30) Expression of Rab38 at the mRNA and protein level was observed in proximal tubule cells of wild-type rats.(31) FHH rats, a natural *Rab38* null mutation, show increased urinary albumin excretion without changes in their glomerular permeability.(21) In these animals, the expression of a Brown Norway *Rab38* transgene led to phenotypic rescue, and knockdown of *Rab38* in a proximal tubule cell system significantly decreased albumin endocytosis.(22) Together, these observations support an important role for the small Rab GTPase RAB38 in the reabsorption of filtered albumin.

Impaired RAB38 function may lead to increased albumin excretion via different mechanisms: altered intracellular vesicle transport may affect albumin reabsorption or recycling of reabsorbed albumin back to the plasma membrane.(32) Alternatively, altered RAB38 function may affect the delivery of proteins required for albumin endocytosis such as cubilin or megalin, the mechanism underlying albuminuria in Dent's disease.(33) Our experimental data showing

reduced abundance of cubilin and megalin in *Rab38* KO but not control rats is consistent with the latter hypothesis. Finally, it is also conceivable that impaired RAB38 function may directly cause glomerular damage, in turn leading to increased concentrations of urinary albumin.

Although the combined evidence from *Rab38* KO rats along with the gene expression and GWAS data strongly implicate *RAB38* as the gene underlying albuminuria in humans, the intergenic index SNP mapped upstream of *RAB38* and downstream of *CTSC* and was found to associate with transcript levels of both genes in whole blood. We can therefore not exclude the possibility that *CTSC* may be the causal gene underlying the observed associations, or that it contributes to the phenotype in addition to *RAB38*. *CTSC* encodes for a lysosomal cysteine protease. Rare mutations in the gene cause autosomal-recessive Papillon-Lefevre syndrome. No renal abnormalities have been reported in affected patients,(34) *Ctsc* KO mice do not show kidney abnormalities,(35) and the gene has not been linked to albuminuria or kidney disease.

The other genomic locus associated with albuminuria in diabetes contains *HS6ST1*, encoding the enzyme heparan sulfate (HS) 6-O-sulfotransferase that catalyzes the 6-O-sulfation of HS and heparin.(36,37) HS are anionic side-chains of HS proteoglycans, which are components of basement membranes, extracellular matrix and cell surfaces. Several studies have reported that inactivation or removal of HS lead to proteinuria, and biopsies from diabetic patients revealed changes in HS sulfation patterns compared with controls.(38) Thus, a genetic variant altering the enzyme's activity or abundance may lead to altered albuminuria. The underlying mechanisms could be manifold, as HS have been reported to not only impact glomerular filtration but also affect growth factor signaling, composition and functions of the glomerular basement membrane, and functions at the endothelial surface layer(38) and the proximal tubule.(39)



Strengths of our study include its large sample size, specific examination of individuals with diabetes, and consistent effects across a variety of studies underscoring the relevance of our findings at the general population level. In addition, we performed careful characterization of a replicated finding through *in vivo* experiments in *RAB38* KO and control rats that cannot, however, elucidate the exact mechanism by which genetic variation at this locus influences albuminuria in humans. Limitations include the fact that the replicated SNPs did not achieve genome-wide significance necessitating future confirmation in even larger studies, and that we could not assess allele-specific gene expression in human kidney tissues. We focused on European Ancestry study participants and mostly on individuals with type 2 diabetes. Future studies should therefore examine these associations among individuals of additional ancestries and in well-powered studies of patients with type 1 diabetes. Although results were combined after study-specific analyses, biological variation in UACR and different urine collection and storage methods may have resulted in increased variation and thus reduced statistical power to reveal significant associations. Additional studies are required to determine the causal variants and the exact underlying molecular mechanism by which genetic variation at *RAB38/CTSC* and *HS6ST1* associates with albuminuria in humans. An elucidation of the underlying mechanisms and the contributions and differences of albuminuria of glomerular and tubular origin may improve our understanding of proteinuric kidney diseases in general, but may be especially relevant to DKD, the most common cause of end-stage renal disease.

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#### KORA-F3/KORA-F4 Study

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#### LIFELINES Study

The LifeLines Cohort Study, and generation and management of GWAS genotype data for the LifeLines Cohort Study is supported by the Netherlands Organization of Scientific Research NWO (grant 175.010.2007.006), the Economic Structure Enhancing Fund (FES) of the Dutch government, the Ministry of Economic Affairs, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the Northern Netherlands Collaboration of Provinces (SNN), the Province of Groningen, University Medical Center Groningen, the University of Groningen, Dutch Kidney Foundation and Dutch Diabetes Research Foundation. LifeLines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviours of 165,000 persons living in the North East region of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics.

MESA Study

University of Washington (N01-HC-95159), Regents of the University of California (N01-HC-95160), Columbia University (N01-HC-95161), Johns Hopkins University (N01-HC-95162, N01-HC-95168), University of Minnesota (N01-HC-95163), Northwestern University (N01-HC-95164), Wake Forest University (N01-HC-95165), University of Vermont (N01-HC-95166), New England Medical Center (N01-HC-95167), Harbor-UCLA Research and Education Institute (N01-HC-95169), Cedars-Sinai Medical Center (R01-HL-071205), University of Virginia (subcontract to R01-HL-071205).

MICROS Study

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PREVEND Study

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SAPHIR Study

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SHIP/SHIP-Trend/GANI MED Study

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#### SKIPOGH Study

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#### Vanderbilt Study

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#### Susztak Laboratory

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#### Jacobs Laboratory

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#### Devuyst Laboratory

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#### Additional Data Resources

Data on glycemic traits have been contributed by MAGIC investigators and have been downloaded from [www.magicinvestigators.org](http://www.magicinvestigators.org)

Findings from this study were presented at the 51st Congress of the ERA-EDTA in Amsterdam, 2014, and at the CHARGE Investigator Meeting July 2015 in Jackson, MS. An abstract of this work was accepted for platform presentation at the ASHG 2015 Annual Meeting in Baltimore, MD.

#### **Author Contributions**

B.S., C.H., J.T., P.H., G.E., L.L., T.B.H., V.G., A.K., A.D.P., N.J.W., C.S.F., B.K.K., P.S.W., A.L., G.G., Ch.M., C.G., H.E.W., P.P.P., M.J.H., H.J.J., J.L., B.P., H.V., M.N., R.R., R.B., J.C.D. and R.J.C. designed this study.

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G.N., M.J.H., J.L., B.K., B.P., F.K., L.K., S.C., H.V., R.R., U.V., N.E., U.L., B.Po., D.A., G.B.E. and M.P. were involved in the study management.

C.H., P.H., G.E., V.G., A.S., J.C., O.D., O.P., N.J.W., C.S.F., B.K.K., P.S.W., A.L., G.G., Ch.M., C.G., H.E.W., J.L.H., Pvd.H., R.T.G., P.P.P., B.P., L.K., H.W., H.V., M.N., S.S. and R.J.C. recruited the subjects.

J.T., P.H., A.V.S., T.A., Ad.T., Y.L., M.L., J.C., A.K., R.S., A.D.P., C.S.F., M.R., V.M., M.G., B.O.T., C.P., N.C.Y., M.J.H., J.L., B.P., F.K., L.K., S.C., A.T. and K.H.E. interpreted the results.

J.T., P.H., Ad.T., M.L., A.K., Y.K., K.S., M.G., I.M.H., C.A.B., C.P., N.C.Y., M.J.H., H.J.J., J.L., A.T. and K.H.E. drafted the manuscript.

M.M., A.V.S., T.A., J.O., A.P., Ad.T., Y.L., M.L., M.F., A.K., G.L., R.K., R.S., Z.K., C.Ha., L.H., A.D.P., Y.K., K.S., Ji.L., M.H.C., Q.Y., M.O., S.J.H., M.R., C.M., V.M., M.G., I.M.H., C.A.B., B.O.T., S.E.R., D.T., C.F., C.P., N.V., N.C.Y., M.J.H., B.K., A.T., K.H.E., C.M.S., R.J.C. and A.Y.C. developed statistical methods and performed the analyses.

A.S., B.D.M., E.B., C.Ha., O.P., C.L., R.J.F.L., M.R., T.Z., N.S., H.G., M.Wa., T.I., A.M.Z., M.H., S.C., G.H. and U.V. performed the genotyping.

J.O., Y.L., Y.K., K.S., Ji.L., C.M., V.M., G.M., M.G., I.M.H., C.A.B., Pvd.H., D.T., N.V. and C.M.S. conducted the bioinformatics analyses.

N.C.Y., A.L., A.M.Z., M.J.H., O.D., H.J.J. and J.L. did the animal work or provided functional data. All authors critically reviewed the manuscript.

### Conflict of Interest Statement

C.H. received Honoraria from Novartis. J.Ch., J.T., P.H. received research grants and honoraria from Servier. M.W. had consultancies with Amgen and Novartis and received support from Sanofi; M.W. did not participate in the animal experiments. K.S. received research support from Boehringer Ingelheim and was on advisory board of Abbvie. A.T., Ad.T., R.S., M.G., N.C.Y., A.Y.C., M.L., Y.L., V.M., Y.K., D.T., A.L., M.H.C., Q.Y., M.F., M.O., L.H., B.O.T., C.F., A.K.D., A.S., A.V.S., A.M.Z., A.L., B.K., B.Po., B.S., B.K.K., B.P., B.D.M., C.Ha., C.H., Ch.M., C.G., C.M.S., C.M., C.L., D.A., D.S., E.B., F.K., G.B.E., G.H., G.W., G.N., G.G., G.M., G.E., G.L., H.E.W., H.G., H.W., H.V., H.B., H.K., I.M.L., I.R., J.L.H., J.S.B., J.C.L., Ji.L., J.H.Z., J.C., J.C.D., K.B., L.J.L., L.F., L.K., M.H., M.M., M.J.H., M.N., M.Wa., M.P., M.B., M.R., N.V., N.J.W., N.E., N.S., O.P., Pvd.H., P.P.P., P.V., P.S.W., R.T.G., R.R., R.B., R.J.C., R.K., R.J.F.L., S.J.H., S.C., S.B., S.E.R., S.S., T.B.H., T.C., T.Z., T.I., T.A., T.T., U.L., U.V., V.G., V.C., W.K., Z.K., J.R.O., A.P., I.M.H., A.D.P., I.Hd.B., O.D., J.L., K.H.E., H.J.J., C.A.B., C.S.F., C.P., and A.K. did not report any potential conflicts of interest.

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Tables

**Table 1:** Replicated SNP associations with UACR in individuals with diabetes

	sample size	effect on log(UACR[mg/g])	s.e.	p-value	$I^2$ %
<b>rs649529, <i>RAB38</i></b>					
discovery	5825	-0.15	0.03	9.3E-06	0
replication	1962	-0.12	0.05	0.02	0
combined	7787	-0.14	0.03	5.8E-07	0
<b>rs13427836, <i>HS6ST1</i></b>					
discovery	5509	0.20	0.04	6.1E-06	10
replication	1890	0.16	0.07	0.03	58
combined	7399	0.19	0.04	6.3E-07	30

For both variants, the effect of each additional copy of the minor allele (T) on UACR was modeled in an additive fashion.  $I^2$  is provided as a measure of heterogeneity across studies. Imputation quality ranged from 0.41 to 1.0 for rs649529 and from 0.44 to 1.0 for rs13427836. The variants were directly genotyped in four of the replication studies, with a call rate ranging from 0.98 to 1 for rs649529 and of 0.99 for rs13427836. s.e.: standard error. The estimated proportion of explained variance in UACR among those with diabetes is 0.6% for rs649529 and 0.5% for rs13427836, using the formula  $2 \times \text{MAF} \times (1 - \text{MAF}) \times \text{effect}^2 / \text{var}(\log[\text{UACR}])$ , based on the combined effect estimates from Table 1 and the phenotypic variance in the large population-based ARIC Study.

**Table 2:** Replicated SNP associations with additional kidney function and diabetes-related traits

<b>rs649529, RAB38/CTSC</b>					
<b>trait</b>	<b>n</b>	<b>effect (OR)</b>	<b>s.e.</b>	<b>p-value</b>	<b>p-difference*</b>
<b>UACR, diabetes; log(mg/g)</b>	7787	-0.14	0.03	5.8E-07	6.9E-06
UACR, no diabetes; log(mg/g)	45094	-0.004	0.008	0.64	
<b>eGFRcrea, diabetes; log(ml/min/1.73m<sup>2</sup>)</b>	11527	0.003	0.004	0.46	2.8E-01
eGFRcrea, no diabetes; log(ml/min/1.73m <sup>2</sup> )	118427	-0.001	0.001	0.59	
<b>CKD (eGFR&lt;60 ml/min/1.73m<sup>2</sup>)</b>	118114	(1.01)	0.02	0.57	
<b>Type 2 diabetes</b>	63390	(1.02)	0.02	0.32	
<b>Fasting Glucose; (mmol/l)</b>	46186	0.003	0.006	0.65	
<b>HbA1c; (%)</b>	46368	0.004	0.004	0.31	
<b>rs13427836, HS6ST1</b>					
<b>trait</b>	<b>n</b>	<b>effect (OR)</b>	<b>s.e.</b>	<b>p-value</b>	<b>p-difference*</b>
<b>UACR, diabetes; log(mg/g)</b>	7399	0.19	0.04	6.3E-07	1.7E-05
UACR, no diabetes; log(mg/g)	34830	0.010	0.012	0.38	
<b>eGFRcrea, diabetes; log(ml/min/1.73m<sup>2</sup>)</b>	11092	0.008	0.006	0.13	1.3E-01
eGFRcrea, no diabetes; log(ml/min/1.73m <sup>2</sup> )	114247	0.000	0.001	0.94	
<b>CKD (eGFR&lt;60 ml/min/1.73m<sup>2</sup>)</b>	113612	(0.97)	0.02	0.23	
<b>Type 2 diabetes</b>	63390	(1.00)	0.03	0.94	
<b>Fasting Glucose; (mmol/l)</b>	46186	-0.005	0.004	0.22	
<b>HbA1c; (%)</b>	46368	0.003	0.005	0.61	
<b>rs10795433, CUBN†</b>					
<b>trait</b>	<b>n</b>	<b>effect (OR)</b>	<b>s.e.</b>	<b>p-value</b>	<b>p-difference*</b>
<b>UACR, diabetes; log(mg/g)</b>	5825	0.19	0.04	2.0E-05	8.2E-04
UACR, no diabetes; log(mg/g)	46061	0.045	0.01	8.7E-06	
<b>eGFRcrea, diabetes; log(ml/min/1.73m<sup>2</sup>)</b>	11522	0.007	0.005	0.18	0.19
eGFRcrea, no diabetes; log(ml/min/1.73m <sup>2</sup> )	118299	0.0007	0.001	0.61	
<b>CKD (eGFR&lt;60 ml/min/1.73m<sup>2</sup>)</b>	118121	(1.04)	0.02	0.08	
<b>Type 2 diabetes</b>	63390	(1.00)	0.03	0.88	
<b>Fasting Glucose; (mmol/l)</b>	46186	-0.003	0.005	0.52	
<b>HbA1c; (%)</b>	46368	-0.002	0.005	0.73	

Effects represent the change in trait associated with each additional copy of the minor allele for each of the SNPs. For continuous traits, units are provided; the effect for binary outcomes, shown in parentheses, represents an odds ratio (OR). Estimates refer to the discovery samples of the respective trait and to the published resources for the glycemic traits. Fasting glucose and HbA1c were evaluated among individuals free of diabetes. For the kidney traits, p-values and standard errors are corrected using genomic control. \*P-value for difference from a two-sample t-test:  $t = (\text{effect}_{\text{DM}} - \text{effect}_{\text{nonDM}}) / (\text{s.e.}_{\text{DM}}^2 + \text{s.e.}_{\text{nonDM}}^2)$  which, for large sample sizes is distributed as a Normal (0,1). The correlation between  $\text{effect}_{\text{DM}}$  and  $\text{effect}_{\text{nonDM}}$  is assumed to be 0. s.e.: standard error. †Effect estimates for *CUBN* are provided from the discovery stage.

Associations with type 2 diabetes were tested using the publicly available summary statistics dataset from the DIAGRAM Consortium (12,171 cases and 56,862 controls).(40) Associations with fasting glucose and plasma hemoglobin A1c concentrations were evaluated using the publicly available results from the MAGIC Consortium ([www.magicinvestigators.org](http://www.magicinvestigators.org)).(41,42)

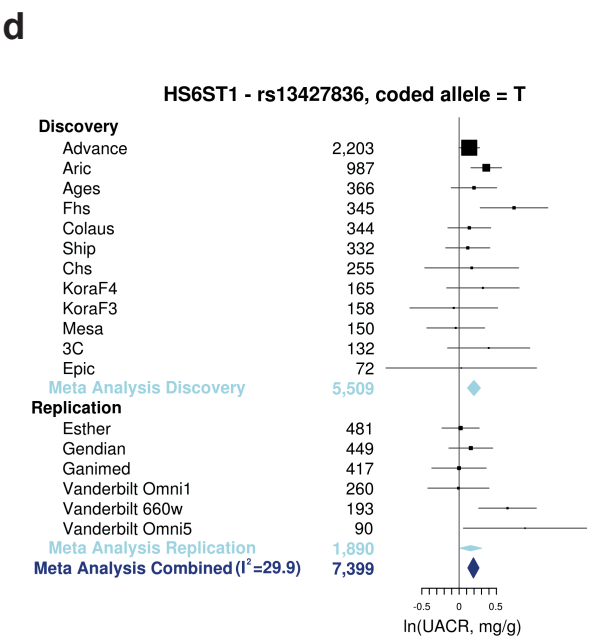
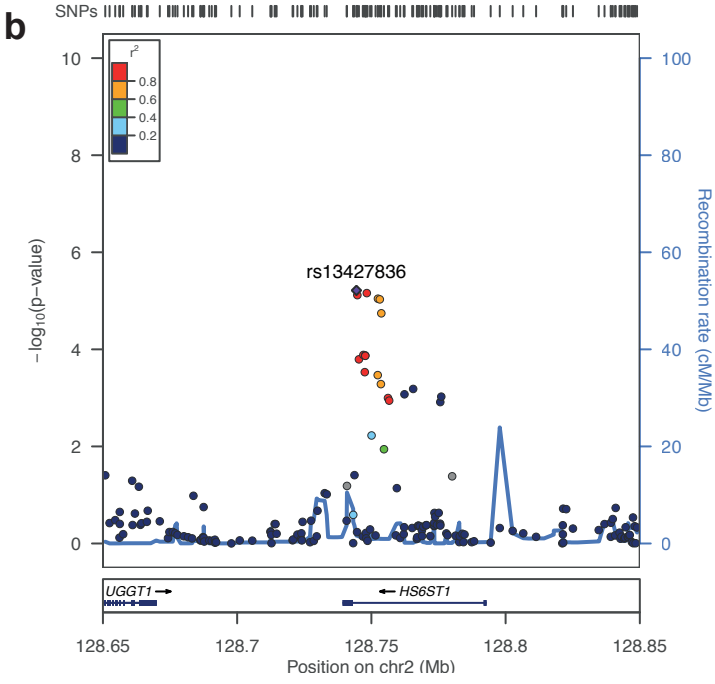
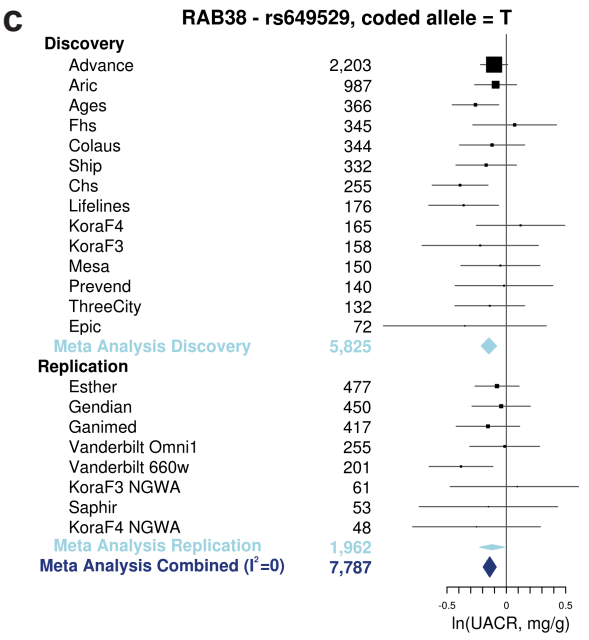
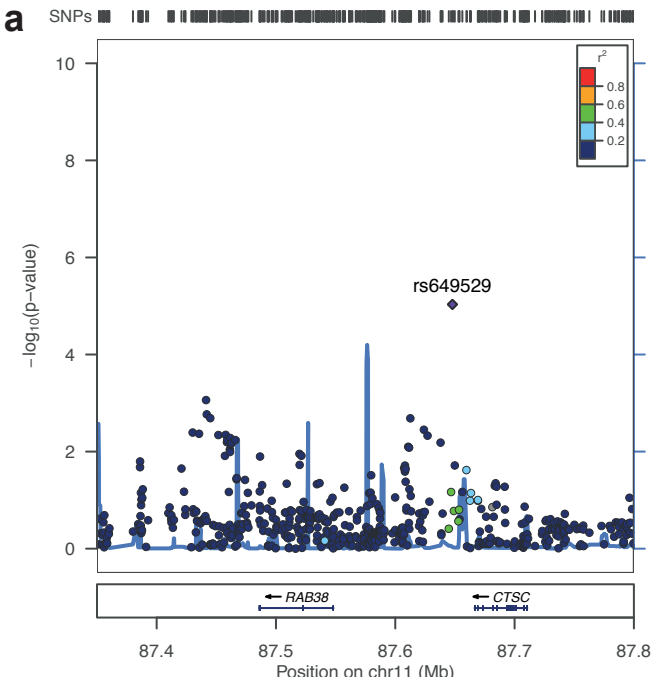


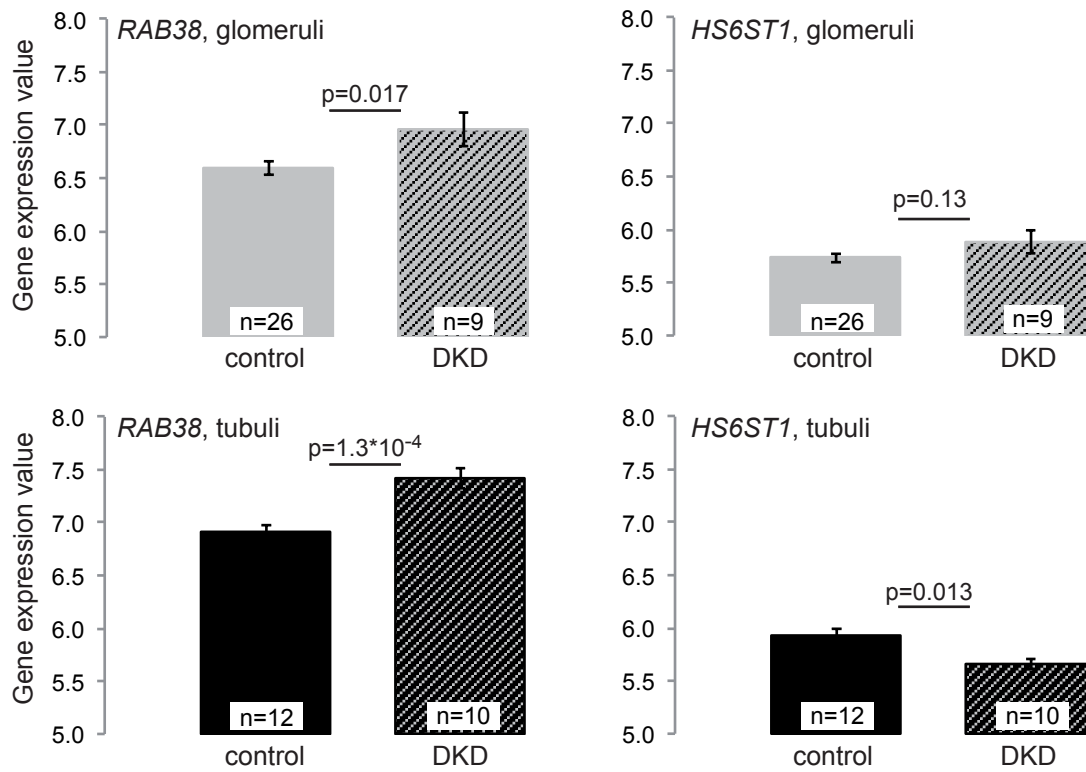
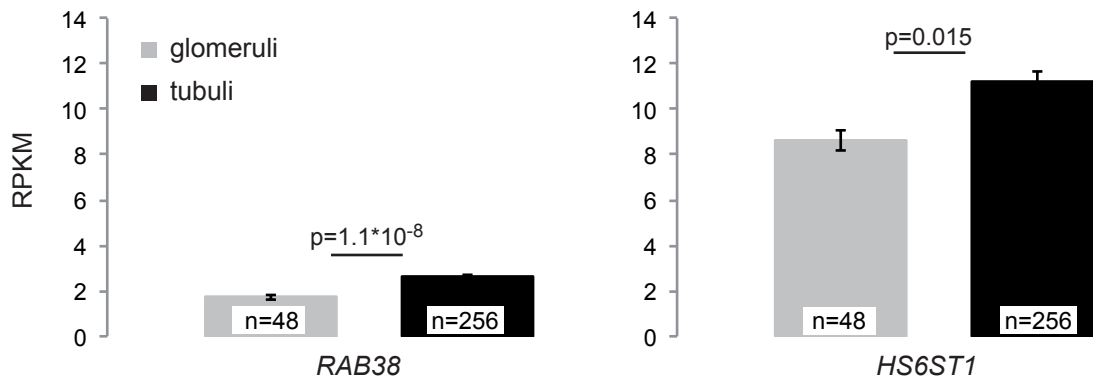
## Figure Legends

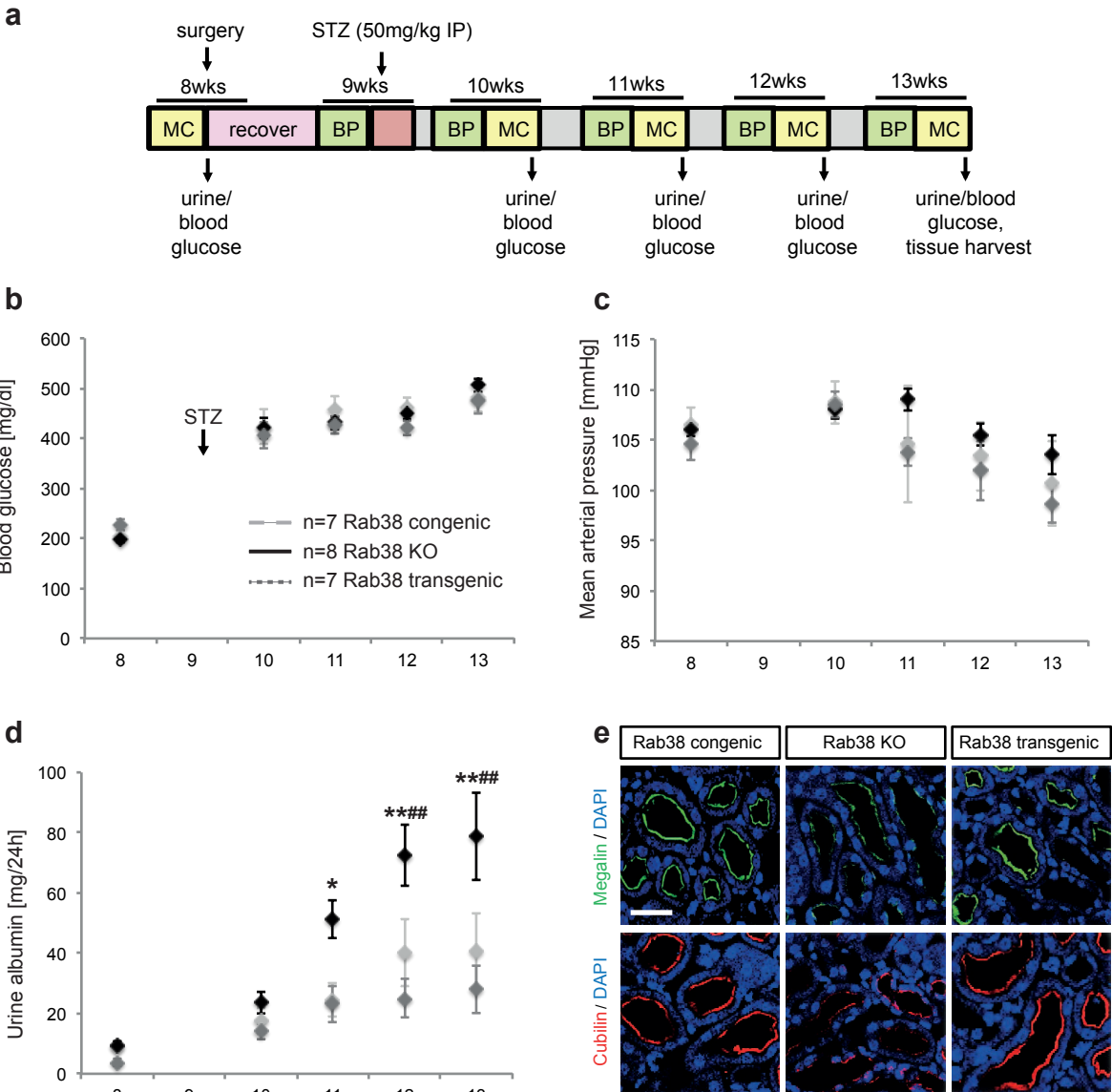
**Figure 1: Overview of associated genomic loci at *RAB38/CTSC* and *HS6ST1* and consistent association with albuminuria in diabetes across the contributing studies.** (a) Regional association plot of the *RAB38/CTSC* locus on chromosome 11 (b) The T allele at rs649529 is associated with lower UACR across discovery and replication studies (c) Regional association plot of the *HS6ST1* locus on chromosome 10 (d) The T allele of intronic rs13427836 is associated with higher UACR across discovery and replication studies.

**Figure 2: *RAB38* and *HS6ST1* expression across kidney tissues.** (a) Comparison of *RAB38* and *HS6ST1* expression (microarray) in tubuli and glomeruli of patients with DKD and controls shows significantly higher *RAB38* expression in tubuli of DKD patients than in tubuli of controls (significance threshold  $0.05/6=8.3 \times 10^{-3}$  for investigating *RAB38*, *CTSC* and *HS6ST1* in tubuli and glomeruli). *CTSC* expression was not significantly different between DKD cases and controls in tubuli ( $p=0.11$ ) or glomeruli ( $p=0.03$ ). Expression levels are shown as RMA-processed gene intensity values. Error bars correspond to the standard error of the mean (s.e.m.). (b) *RAB38* and *HS6ST1* transcript abundance quantified from RNA-seq is detected at high levels in human tubuli but also in glomerular cells. Transcripts were quantified by reads per kilobase of transcript per million mapped (RPKM). Error bars correspond to the standard error of the mean (s.e.m.).

**Figure 3: Comparison of *Rab38* congenic, transgenic and KO rats after induction of diabetes.** (a) Experimental setup and timeline (b) Comparison of blood glucose concentrations (c) Comparison of mean arterial pressure (d) Comparison of urinary albumin concentrations (e) Expression of endocytic markers. Immunofluorescence staining for megalin (green, top panel) and cubilin (red, bottom panel) in kidneys from all three rat strains. Nuclei counterstained with DAPI (blue). Scale bar, 50  $\mu\text{m}$ . Data are presented as mean  $\pm$  standard error of the mean (SEM). The results for blood pressure measurement, urinary albumin excretion, and blood glucose were analyzed by two-way ANOVA followed by Tukey's post hoc test.



**a Comparison of Relative Gene Expression, DKD Patients and Controls****b Comparison of Gene Expression (RNAseq) Across Tissues**



# **Genome-wide Association Studies Identify Genetic Loci Associated with Albuminuria in Diabetes**

## **SUPPLEMENTAL MATERIALS**

This work is dedicated to the memory of our colleague Dr. Wen Hong Linda Kao, a wonderful person, brilliant scientist and central member of the CKDGen Consortium.

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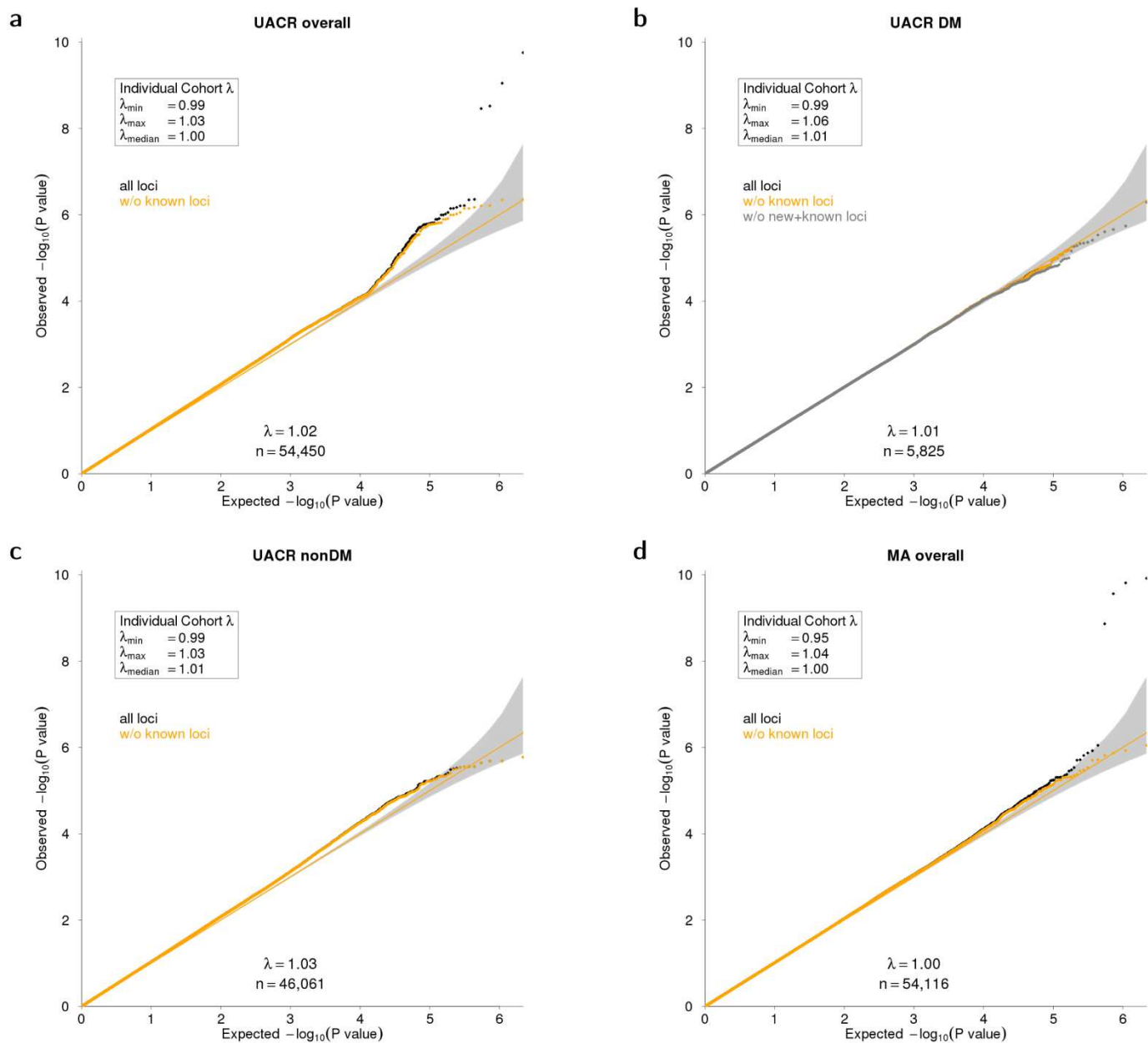
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### Supplementary Figure 1: QQ plots for all GWAS meta-analyses

Quantile-quantile (QQ) plots of the GWAS meta-analysis results for (a) the urinary albumin-to-creatinine ratio (UACR) in the overall sample, (b) UACR among those with diabetes (c) UACR among those without diabetes, and (d) microalbuminuria (MA) in the overall sample. The observed p-values are plotted on the y-axis against their expected distribution under the null hypothesis on the x-axis.

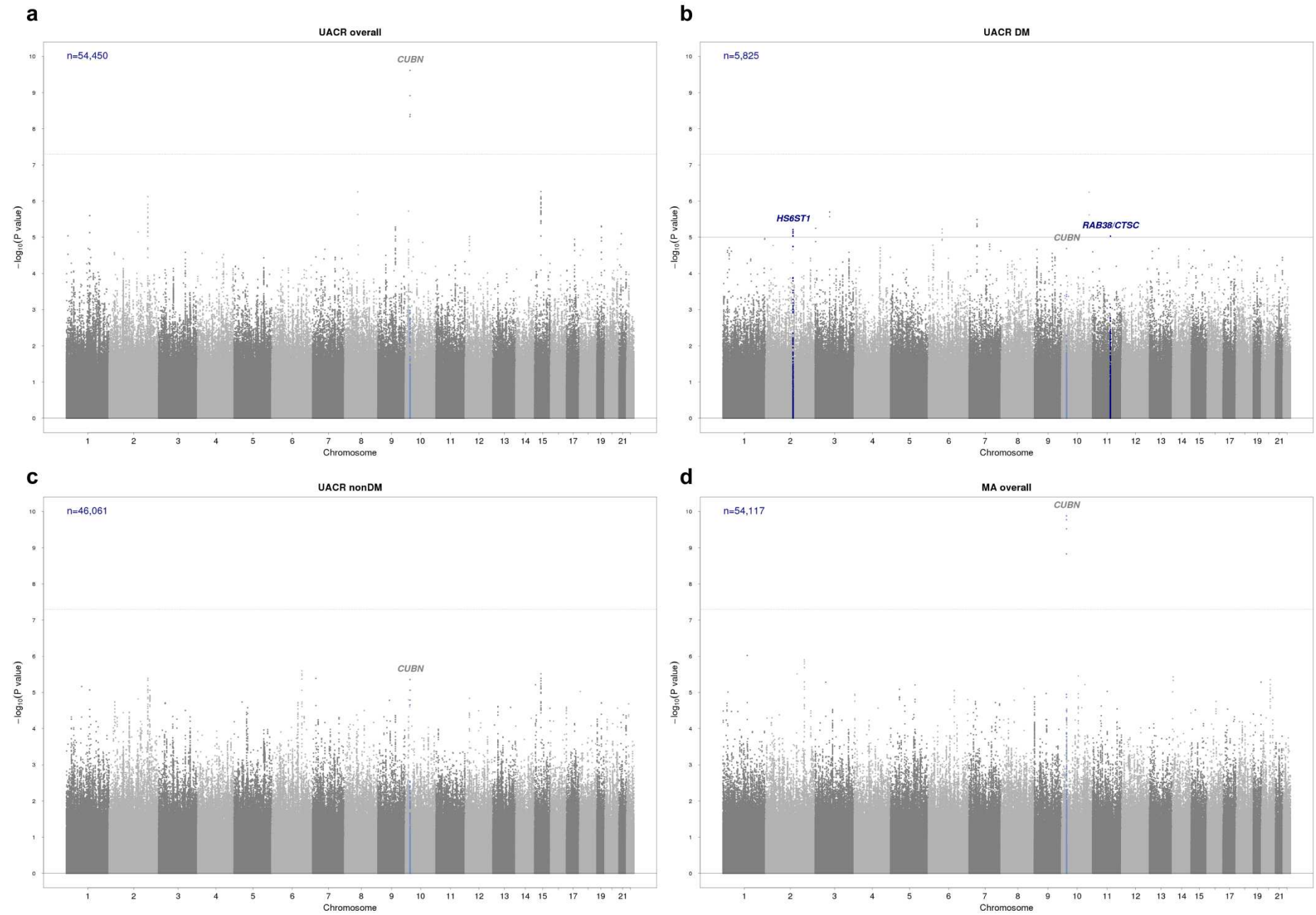


Results for all SNPs are shown in black, and results after removal of loci previously known to contain trait-associated variants are shown in yellow. Gray bands represent 95% confidence intervals.  $\lambda$ : lambda, genomic control parameter;  $n$ : sample size.

**Supplementary Figure 2: Manhattan plots for all GWAS meta-analyses**

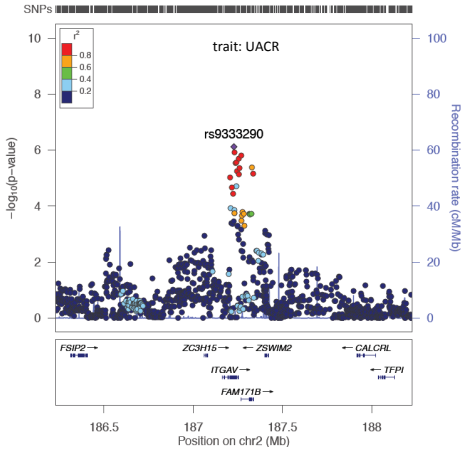
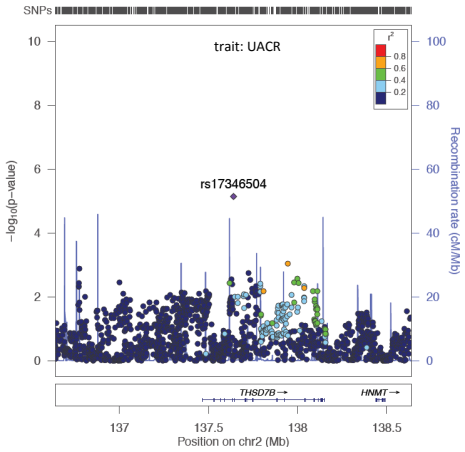
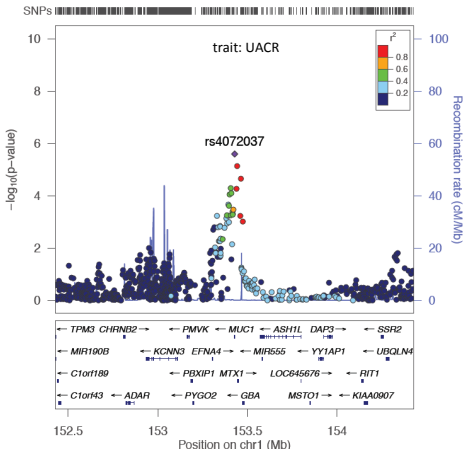
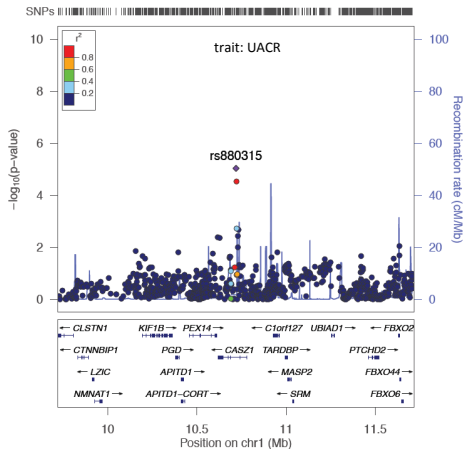
Manhattan plots of the GWAS meta-analysis results for **(a)** UACR in the overall sample, **(b)** UACR among those with diabetes, **(c)** UACR among those without diabetes, and **(d)** microalbuminuria in the overall sample. SNPs are plotted on the x-axis according to their position on each chromosome with the  $-\log_{10}(\text{p-value})$  on the y-axis. The upper solid horizontal line indicates the threshold for genome-wide significance,  $5 \times 10^{-8}$ . The lower solid horizontal line for UACR among those with diabetes **(b)** represents the threshold of  $1 \times 10^{-5}$  applied to select SNPs for replication. Genomic loci previously known to contain trait-associated variants are colored in light blue, new findings in dark blue.

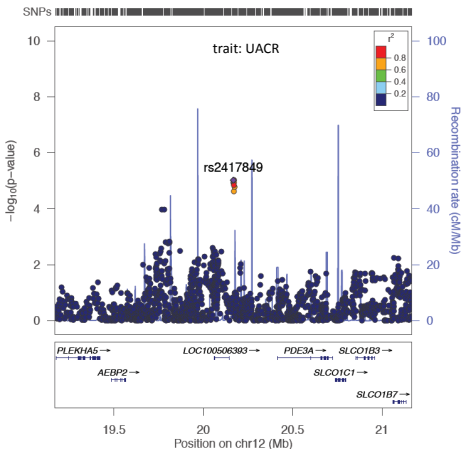
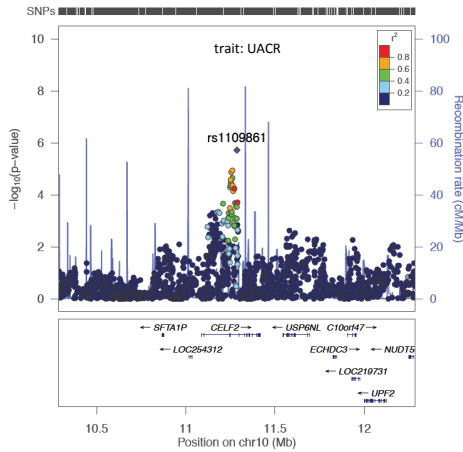
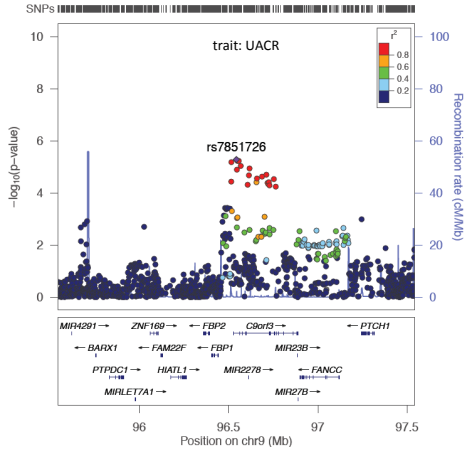
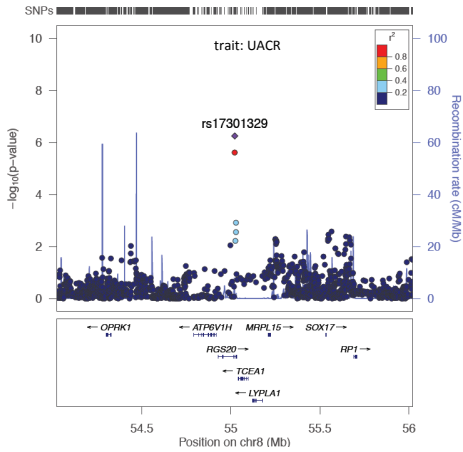


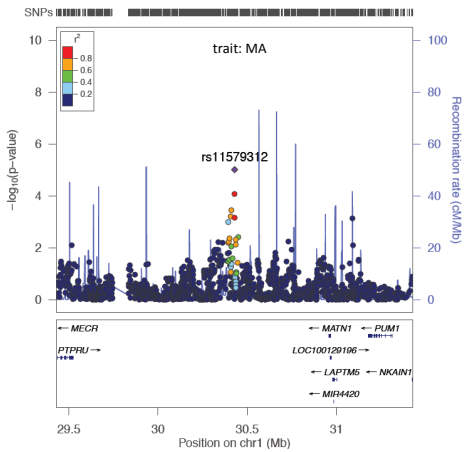
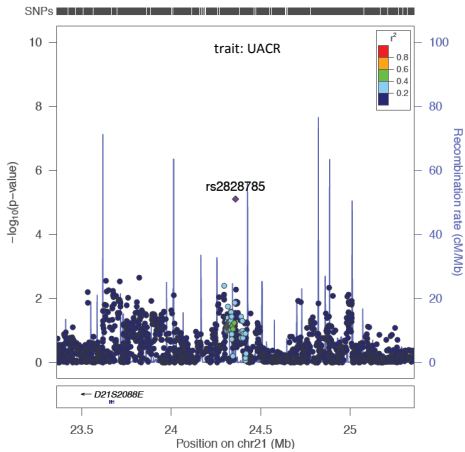
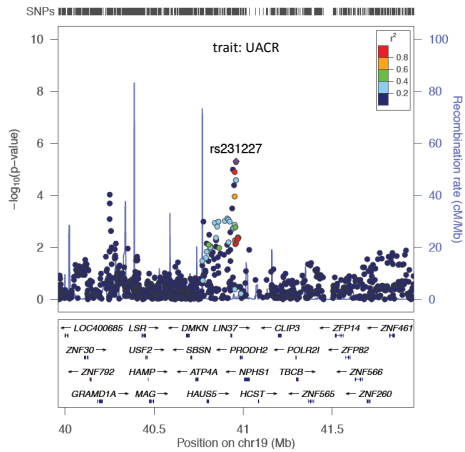
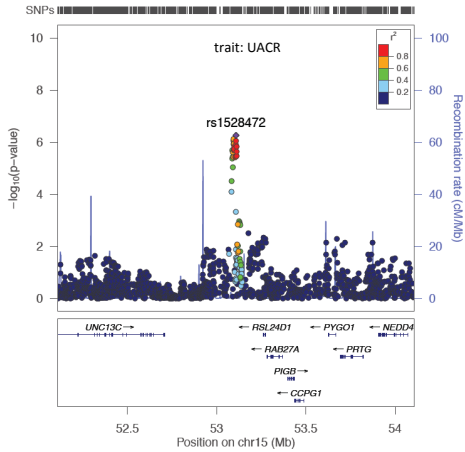


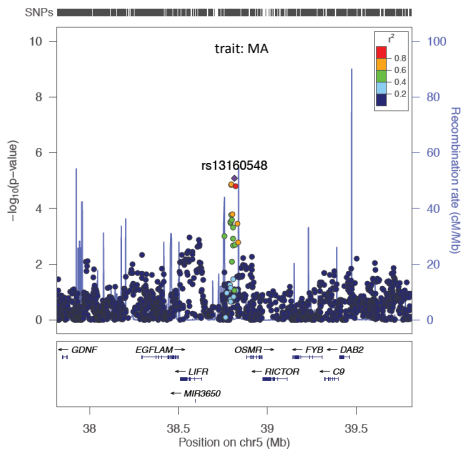
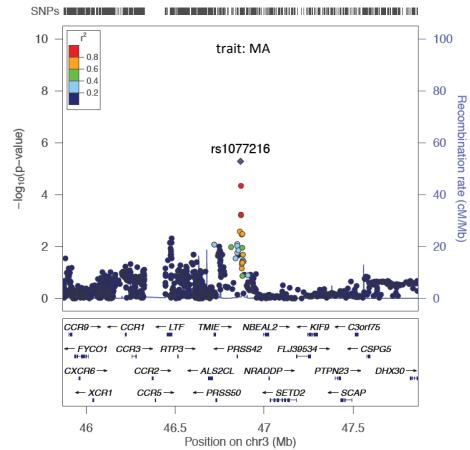
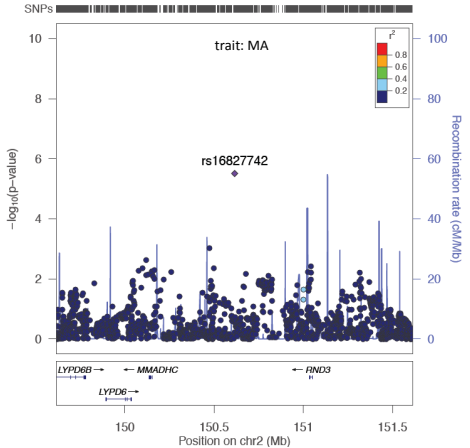
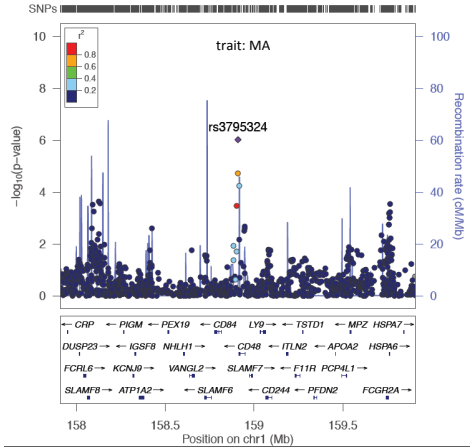
**Supplementary Figure 3: Regional association plots**

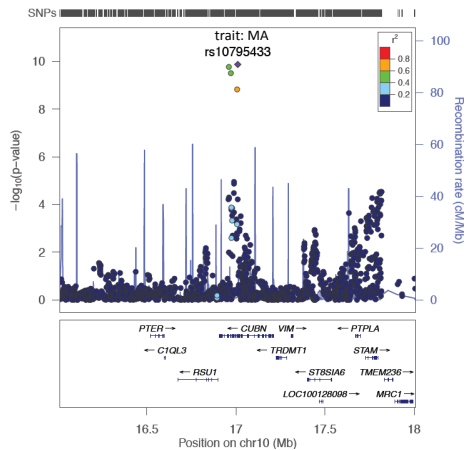
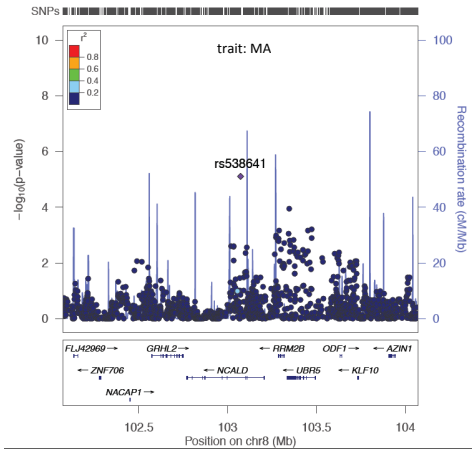
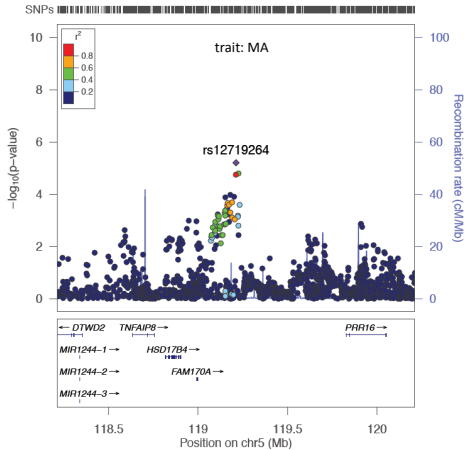
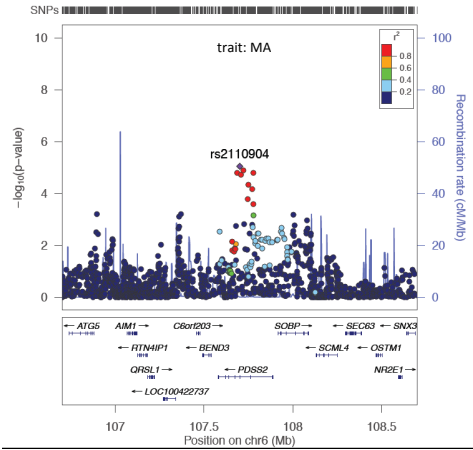
Regional association plots are shown for all loci that contained at least one index SNP associated with the trait at  $p < 10^{-5}$  after correction for genomic control. Correlation with the index SNP is estimated based on the HapMap r22 CEU samples. Plots were generated using the stand-alone version of LocusZoom (Pruim RJ *et al.*, Bioinformatics 2010). When association in a genomic region was observed with more than one trait, the regional association plot of the trait with the lowest p-value is shown. Genetic positions refer to NCBI build 36/hg18 coordinates.

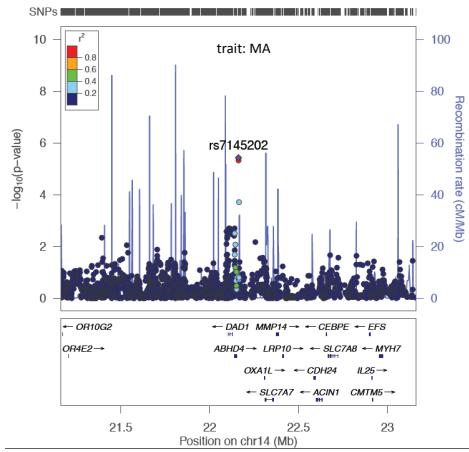
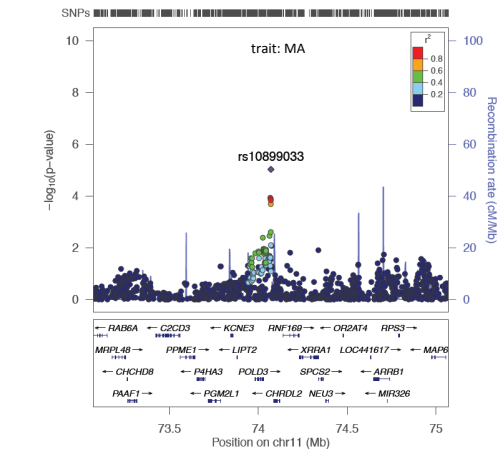
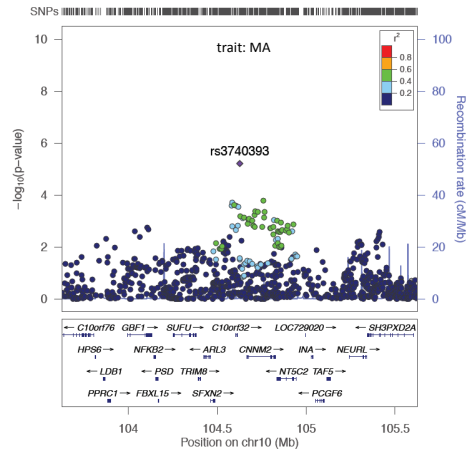
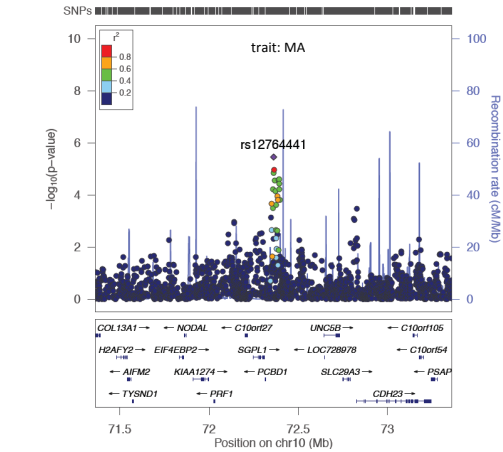




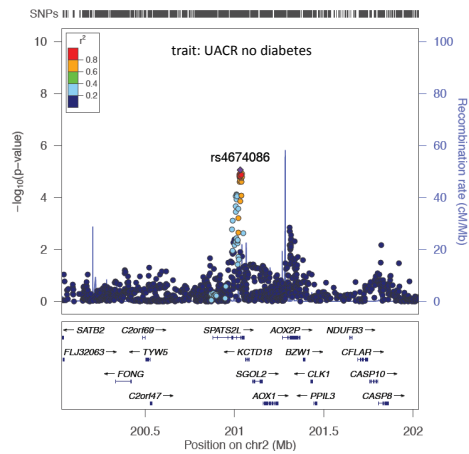
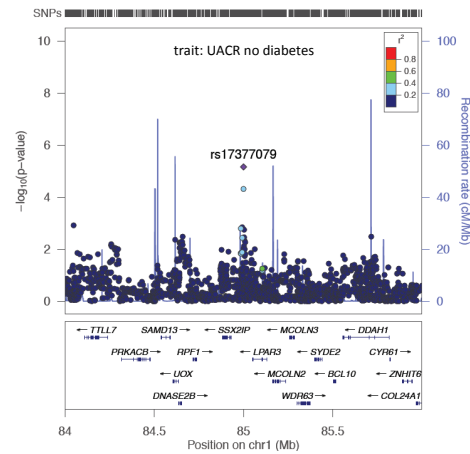
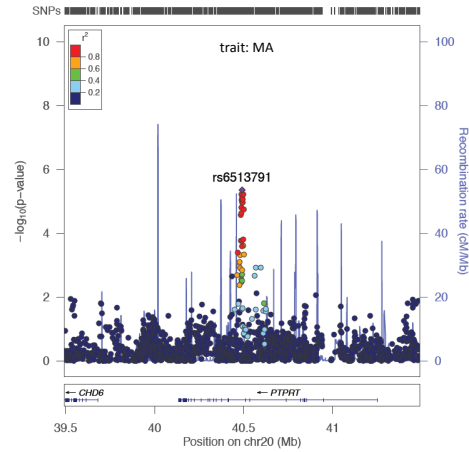
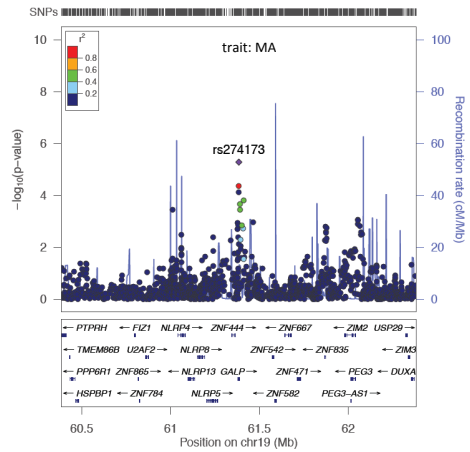


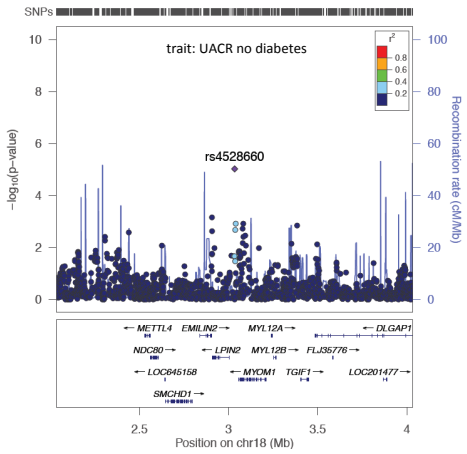
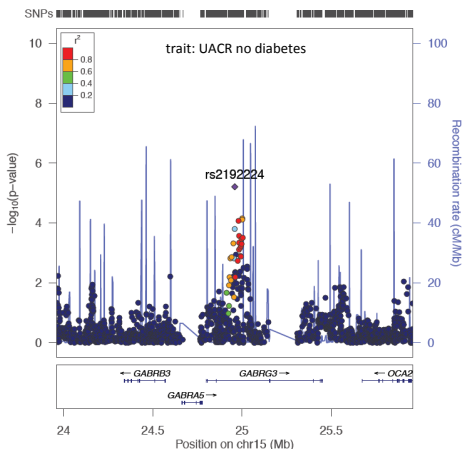
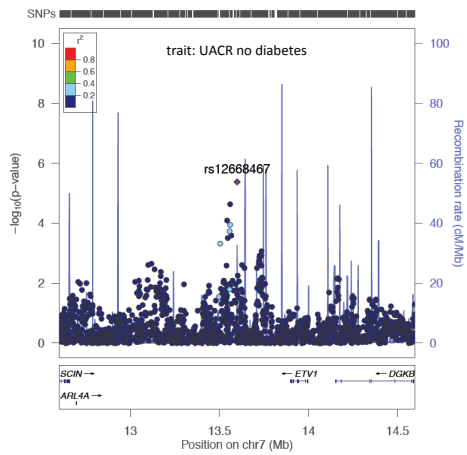
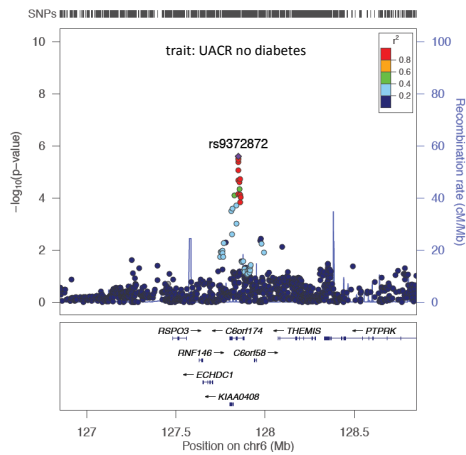


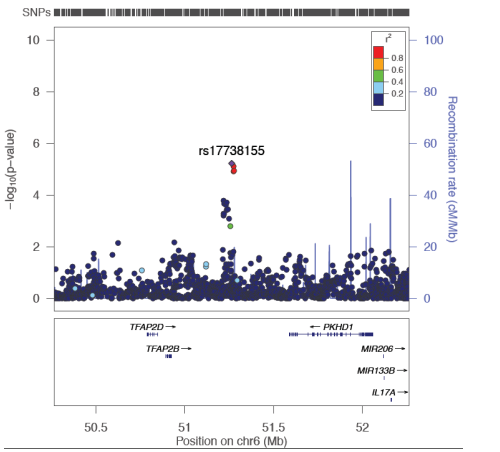
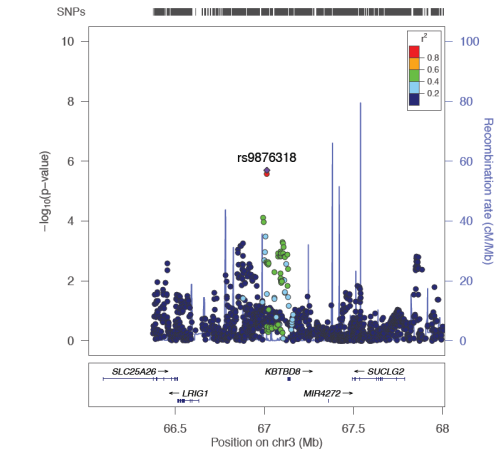
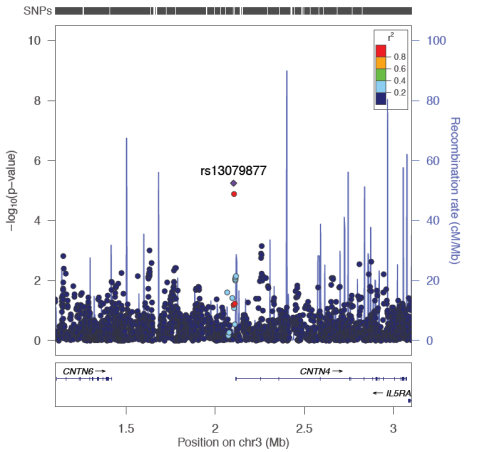
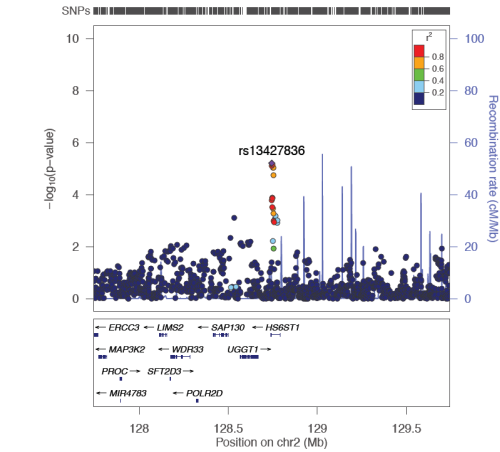


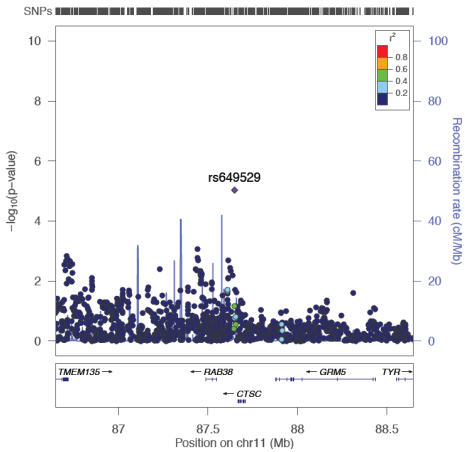
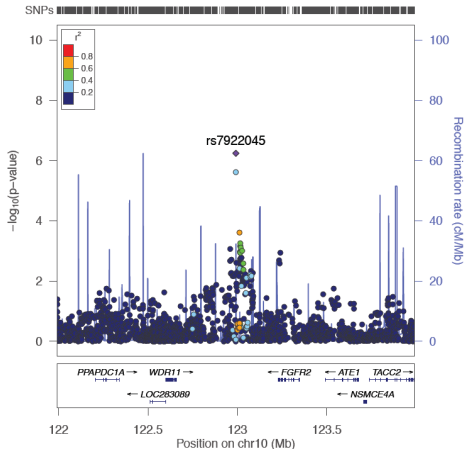
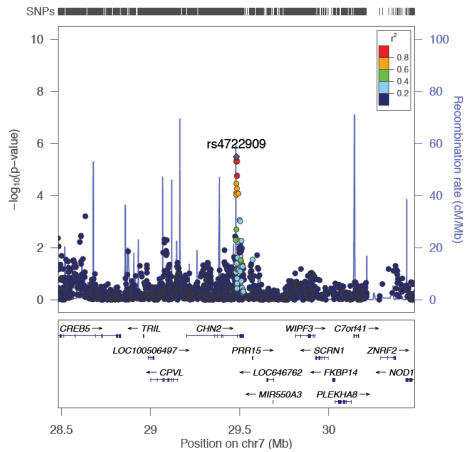






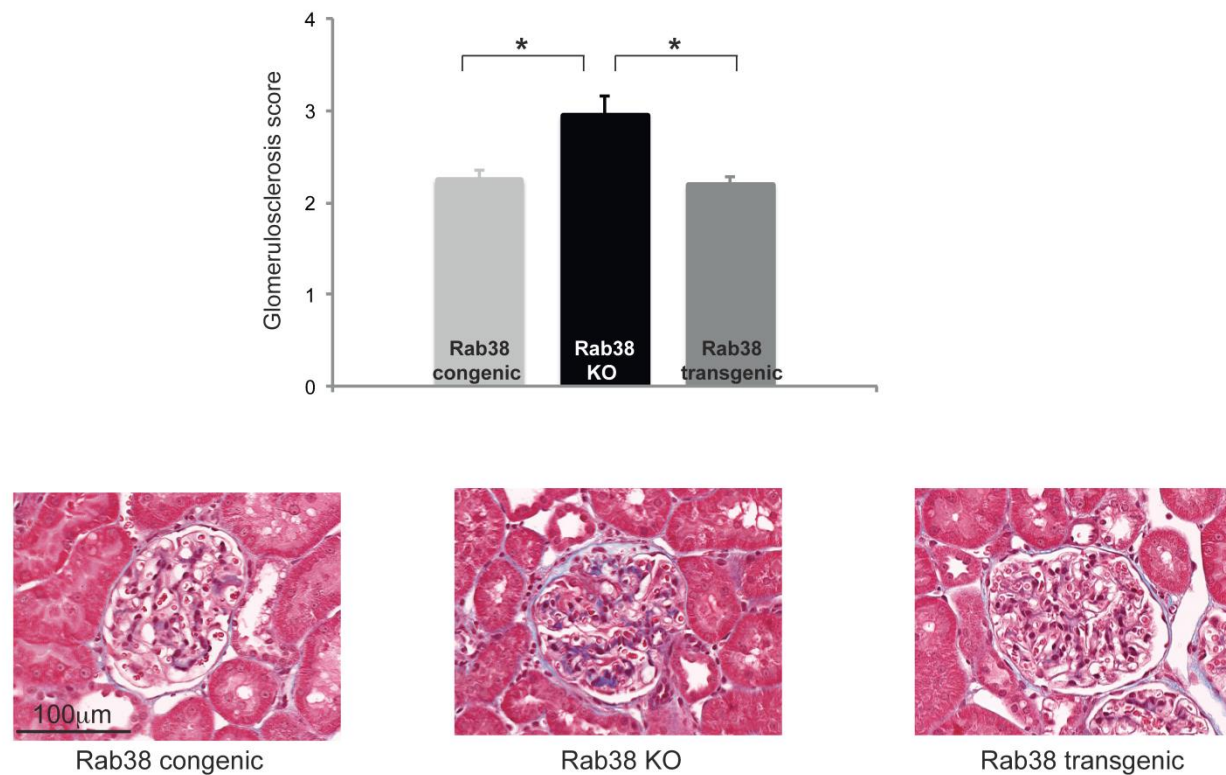






**Supplementary Figure 4: Evaluation of glomerulosclerosis in Rab38 KO, congenic and transgenic rats.**

Representative images of trichrome-stained glomeruli from *Rab38* congenic, KO and transgenic animals. The glomerulosclerosis score was determined from left kidneys of 13-week-old rats (n=3 of each strain) as described previously (O'Meara CC *et al.* JASN, 2011). 50 to 60 40x magnified cortical glomeruli were imaged and scored, and scores were averaged for each animal. \*p<0.05, \*\*p<0.01 KO vs. transgenic, ##p<0.01 KO vs. congenic. Glomerulosclerosis was analyzed using one-way ANOVA followed by Tukey's post hoc test.



Supplementary Table 1: Characteristics of the study populations

Study	UACR sample size	Women, %	Age (years)	eGFR <60 (ml/min/1.73m <sup>2</sup> ) <sup>1</sup>	HTN, %	DM, %	UACR (mg/g) (median, 25th%, 75th%)	MA, %
Discovery cohorts								
3C	1072	63.6	77.8 (4.8)	19.9	74.4	12.3	5.3 (2.6, 10.7)	11.7
Advance	2203	32.8	66.7 (6.76)	14.7	47.6	100	15.6 (6.44, 54.8)	45
AGES	3196	58	76.4 (5.46)	24.2	80.6	11.5	2.66 (1.2, 7.0)	11.9
Amish**	727	48.9	49.5 (16.9)	3.1	18.9	1.7	7 (4.3, 13.5)	NA
ARIC	7243	53.1	61.8 (6.1)	8.7	40.7	14.2	5.3 (3.0, 9.5)	9.4
BLSA**	361	46.1	70.4 (15.2)	17.4	21.9	7.7	7 (4.4, 11.0)	NA
CHS	1865	61.3	71.9 (5.0)	9.5	51.4	11	9.3 (5.3, 19.9)	23
COLAUS	5311	53.2	53.4 (10.8)	3.8	36.1	9.6	5.1 (3.4, 9.1)	9.5
CROATIA-SPLIT**	472	59.8	49.3 (14.65)	5	39.4	5	2.5 (1.3, 5.8)	7.8
EPIC	2371	53.3	59.2 (9.00)	29.87	49.3	3	3.6 (1.5, 8.3)	8.1
Fenland**	1398	56.2	44.9 (7.3)	0.9	18.9	1.4	4.5 (3.2, 7.1)	5.5
FHS	6523	54.3	51.2 (14.0)	10.7	57.5	9.7	4.58 (2.62, 9.89)	9.69
INCIPE**	940	52.7	61.0 (11.0)	8.6	69.6	10.6	NA*	7.4
KORA-F3	1530	50.5	62.5 (10.1)	10.8	41.1	11.1	4.9 (2.1, 11.1)	12.5
KORA-F4	1804	51.3	60.9 (8.9)	7	20.9	9.2	6.1 (3.8, 11.9)	12.5
LIFELINES	8085	57.2	47.4 (11.2)	NA	31.5	2.2	3.12 (2.2, 4.7)	2.4
MESA	2511	52.3	62.67 (10.2)	9.72	38.6	5.99	4.60 (3.10, 8.50)	9.52
MICROS**	504	56.5	46.2 (16.1)	3.8	37.7	4.3	6.0 (4.0, 9.0)	5.4
PREVEND	3634	48.4	49.6 (12.5)	3.3	31.8	3.4	7.9 (5.0, 15.5)	10.2
SHIP	2655	51.7	54.5 (15.3)	7.7	51.1	11.2	8.95 (5.00, 20.59)	25.2
SHIP-TREND**	985	56.2	50.1 (13.7)	4.3	39.6	1.8	6 (3.9, 10.3)	8.5
Total	55390							

Replication cohorts								
ESTHER	2958	55.6	61.87	15.7	57.52	15.87	9.8 (6.2, 19.7)	23.06
GANI_MED	1674	44.0	60.0	36.1	71.2	24.9	11.8 (6.1, 43.9)	37.2
GENDIAN	450	47.1	65.05	32.3	53	100	7.54 (3.57,23.65)	27.6
KORAF4 non-GWAS	1195	52.4	49.2	5.8	13.3	4	5.7 (3.5, 11.4)	23.6
KORAF3 non-GWAS	1389	52.5	51.7	2.6	29.4	5.1	4.4 (1.87, 9.6)	11
SAPHIR	1690	37.1	51.4	6.9	55.7	3.3	3.8 (2.3, 8.3)	9.9
SKIPOGH**	807	52.3	47.1	5.7	22.9	4.5	4.2 (2.7, 7.7)	5.7
Vanderbilt Omni1	472	47.3	54.5	27.7	70.5	18	11.5 (6.0, 39.0)	36.7
Vanderbilt Omni5	144	46.9	50.5	21.7	58.2	33.3	14.5 (6.0, 42.2)	35.4
Vanderbilt 660W	365	56.5	56.5	20.6	57.2	17.9	9.0 (5.0, 26.0)	30.7
Total	<b>11144</b>							

\*Because of the lower detection limit of the assay, the INCIPE Study only contributed to analyses of MA.

\*\*Studies that did not contribute data for analyses of MA or UACR among those with diabetes because of low case numbers.

<sup>1</sup>Timepoint of serum creatinine measurement can differ from that of urinary albumin measurements in some of the studies.

Supplementary Table 2: Information about study design and UACR measurement

Study	Study Design	Total genotyped sample size	Study exclusions or disease enrichment, and data quality control	Urinary albumin measurements + QC	Key Study References
Discovery study					
3C	Prospective population-based	1072	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions.</b> none.	At 4-year follow-up, urinary albumin and creatinine were measured in a fresh morning urine sample in a single laboratory using an immunoturbidimetric assay for albumin and Jaffe method for creatinine.	1. The 3C Study Group. Vascular factors and risk of dementia. Design of the Three-City Study and baseline characteristics of the study population. Neuroepidemiology. 2003; 22:316-325. 2. Lambert J-C, Heath S, Even G, Campion D, Slegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr B, Pasquier F, Fiévet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck F, Helisalmi S, Porcellini E, Hanon O, the European Alzheimer’s Disease Investigators, De Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossù P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Galan P, Dartigues J-F, Tzourio C, Gut I, Van Broeckhoven C, Alépérovitch A, Lathrop M, Amouyel P. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet. 2009;41:1094-9.



Advance	Randomized controlled trial	2203	<b>Study exclusions or disease enrichment:</b> multicenter trial done by 215 collaborating centres in 20 countries, including 11,140 type 2 diabetes subjects all of Caucasian origin. <b>Exclusions:</b> 8829 with no genotype; 10 samples excluded due to sex mismatch, high sample missingness or having <0.8 of Caucasian ethnicity (STRUCTURE 2.3). Of the 2301 remaining samples of good genotype quality, 98 did not have data for UACR.	Urinary albumin and creatinine were measured in the same morning fresh sample in local certified laboratories using local regulations in 20 countries. Units were harmonized centrally by the George Institute. Two samples were required for the determination of the stage of albuminuria. UACR were repeated every 6 months during a 5-year follow-up.	1. Ninomiya T et al. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. J Am Soc Nephrol. 2009 Aug;20(8):1813-21. 2. Patel A et al for the ADVANCE Collaborative Group. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial). Lancet 2007; 370: 829-40.
AGES	Population-based	3196	<b>Study information or disease enrichment:</b> none. <b>Exclusions:</b> exclusion criteria included sample failure, genotype mismatch with reference panel, and sex mismatch, resulting in clean genotype data on 3,219 individuals.	Urinary albumin was measured in a morning urine sample using the Tina-quant immunoturbidimetric assay (Roche Diagnostics, Mannheim). The intra-assay CV was 7.2%. Urinary creatinine in the same samples was measured using the HiCo Creatinine Jaffe method (Roche Diagnostics, Mannheim). The intra-assay CV was 4.2%.	Harris TB, Launer LJ, Eiriksdottir G, Kjartansson O, Jonsson PV, Sigurdsson G, Thorgeirsson G, Aspelund T, Garcia ME, Cotch MF, Hoffman HJ, Gudnason V. Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. Am J Epidemiol. 2007 May 1;165(9):1076-87.
Amish	Population-based "founder" cohort	727	<b>Study information or disease enrichment:</b> none. <b>Exclusions:</b> age < 20, severe chronic disease, call rate < 95%.	Urinary albumin concentration was measured from stored samples using a quantitative immunoturbidimetric assay (Roche Diagnostics, Indianapolis), and creatinine in urine was measured using a modified Jaffe method.	1. Mitchell BD et al. The genetic response to short-term interventions affecting cardiovascular function: rationale and design of the Heredity and Phenotype Intervention (HAPI) Heart Study. Am. Heart J. 155, 823-828 (2008). 2. Ramey SA et al. The association of coronary artery calcification and carotid artery intima-media thickness with distinct, traditional coronary artery disease risk factors in asymptomatic adults. Am. J. Epidemiol. 168, 1016-1023 (2008).

ARIC	Prospective, population-based	7243	<b>Study information or disease enrichment:</b> none. <b>Exclusions:</b> of the 9713 genotyped individuals of European ancestry, we excluded 658 individuals based on discrepancies with previous genotypes, disagreement between reported and genotypic sex, one randomly selected member of a pair of first-degree relatives, or outlier based on measures of average DST or more than 8 SD away on any of the first 10 principal components. Additional samples were excluded for this analysis because of the unavailability of the phenotype.	Using stored specimen from samples collected at visit 4, urinary albumin was measured by a nephelometric method either on the Dade Behring BN100 or on the Beckman Image Nephelometer. Urinary creatinine was measured using the Jaffe method.	The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol. 1989 Apr;129(4):687-702.
BLSA	Population-based	361	<b>Study information or disease enrichment:</b> none. <b>Exclusions:</b> non-European descent or with missing UACR information.	Urinary measurements were conducted on 24-hour urine samples. Urinary albumin was determined with nephelometry (Beckman Array System). Urinary creatinine was measured using a Vitros enzymatic assay (Johnson & Johnson Co., Rochester, NY).	Shock NW et al. Normal Human Aging: The Baltimore Study of Aging. 1984.
CHS	Prospective population-based	1865	<b>Study information or disease enrichment:</b> A total of 1908 persons were excluded from the GWAS study sample due to the presence at study baseline of coronary heart disease, congestive heart failure, peripheral vascular disease, valvular heart disease, stroke or transient ischemic attack or lack of available DNA. <b>Exclusions:</b> The present report is based upon genotyping results from 3,329 CHS Caucasian participants, who were free of clinical cardiovascular disease at baseline, consented to genetic testing, and had DNA available for genotyping. Genotypes were called using the Illumina BeadStudio software. Genotyping was successful in 3,291 persons.	Urinary parameters were measured from a morning urine sample. The albumin was measured by rate nephelometry (Array 360 CE Protein Analyzer, Beckman Instruments, Fullerton, CA). The creatinine was measured using a Kodak Ektachem 700 Analyzer (Eastman Kodak company, Rochester, NY).	1. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A, et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol. 1991;1(3):263-276. 2. Heard-Costa, NL et al. NRXN3 is a novel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. 2009. Plos Genet. 5(6): e1000539.

COLAUS	Population-based	5311	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> samples with call rate < 90% and related individuals.	Urinary albumin was measured using a Bromocresol green assay (Roche Diagnostics, Basel, Switzerland). The inter- and intra-assay CVs were 2.5% and 0.4%. Urinary creatinine was measured using a Jaffe kinetic compensated method. The inter- and intra-assay CVs were 2.9% and 0.7%.	Firmann M, Mayor V, Vidal PM, Bochud M, Pécoud A, Hayoz D, Paccaud F, Preisig M, Song KS, Yuan X, Danoff TM, Stirnadel HA, Waterworth D, Mooser V, Waeber G, Vollenweider P. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord. 2008 Mar 17;8:6. doi: 10.1186/1471-2261-8-6.
CROATIA-SPLIT	Population-based	472	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> missing UACR levels.	Urinary albumin excretion was measured, in stored urine samples, by an automated assay based on a turbimetric method with automatic calibration and quality control (Synchron CX System, Beckman Coulter).	"10001 Dalmatians" Croatia launches its national biobank Rudan I, Marusić A, Janković S, Rotim K, Boban M, Lauc G, Grković I, Dogas Z, Zemunik T, Vatauvuk Z, Bencić G, Rudan D, Mulić R, Krzelj V, Terzić J, Stojanović D, Puntarić D, Bilić E, Ropac D, Vorko-Jović A, Znaor A, Stevanović R, Biloglav Z, Polasek O. Croat Med J. 2009 Feb;50(1):4-6.
EPIC	Population-based	2371	<b>Study exclusions or disease enrichment:</b> participants taking colchicine, probenecid or allopurinol at 1st, 2nd health checks or 3rd follow-up; gout from hospital discharge ICD10 M10, between 1997-2008. <b>Exclusions:</b> none.	Urinary albumin was measured in spot urine by immunonephelometry using the Nephelometer II analyzer (Dade Behring, Marburg, Germany). The intra-assay CV was 2.91%. Urinary creatinine was measured by means of colorimetry using the Dimension AR Analyzer (Dade Behring Marburg, Germany).	1. Day N et al. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. Br J Cancer 80 Suppl 1, 95-103 (1999). 2. Lee CT et al. Cross-sectional association between fish consumption and albuminuria: the European Prospective Investigation of Cancer-Norfolk Study. Am J Kidney Dis 52, 876-86 (2008).
Fenland	Population-based	1398	<b>Study exclusions or disease enrichment:</b> exclusion criteria for the study were: age<30 or age>55, prevalent diabetes, pregnant and lactating women, inability to participate including terminal illness, psychotic illness, or inability to walk unaided. <b>Exclusions:</b> 102 excluded due to call rate < 95%, heterozygosity check (upper bound 0.2882, lower bound 0.2735), relatedness check and duplicate check.	Using stored samples, urinary albumin was measured by means of immunonephelometry using the Nephelometer II analyzer (Dade Behring, Marburg, Germany; intra-assay CV 2.91%). Urinary creatinine was measured through colorimetry using the Dimension AR Analyzer (Dade Behring Marburg, Germany).	Willer CJ, Speliotes EK, Loos RJ et al. (2009) Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet, 41(1): 25-34.

FHS	Prospective family-based	6523	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> Of the 9,274 participants who underwent genotyping, we made the following exclusions: sample call rate <97% (n=666), genotype heterozygosity > 5 standard deviations, and ambiguous family data (n=127). This resulted in a total of 8,481 genotyped individuals. Of them, 1958 did not have the phenotype available.	Urinary albumin was measured from stored samples using a Tina-quant immunoturbimetric assay (Roche Diagnostics, Indianapolis, Indiana). The intra-assay CV was 7.2% for the Offspring cohort and 2.1% for the Third Generation. Urinary creatinine was measured using a modified Jaffe method. Its intra-assay CV was 2.3% for the Offspring cohort and 1.0% for the Third Generation cohort.	1. Feinleib M, Kannel WB, Garrison RJ, McNamara PM, Castelli WP. The Framingham Offspring Study. Design and preliminary data. Prev Med. 1975;4:518-525. 2. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol. 1979;110:281-290. 3. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino RB, Sr., Fox CS, Larson MG, Murabito JM, O'Donnell CJ, Vasan RS, Wolf PA, Levy D. The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. Am J Epidemiol. 2007;165:1328-1335.
INCIPE	Cross-sectional, population based	940	<b>Study exclusions or disease enrichment:</b> individuals <40 year old. <b>Exclusions:</b> pregnant women	Using stored specimen, urinary albumin was measured by a nephelometric method. Urinary creatinine was measured using the Jaffé method.	Gambaro, G. et al. Prevalence of CKD in northeastern Italy: results of the INCIPE study and comparison with NHANES. Clin. J. Am. Soc. Nephrol. 5, 1946-1953 (2010).
KORA-F3	Prospective population-based	1530	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> none.	Using stored urine samples, urinary albumin concentration was measured with a latex enhanced nephelometric assay (Siemens Healthcare Diagnostics) on a Dade Behring BN2 apparatus. Urinary creatinine concentration was measured using an enzymatic method.	1. Baumeister SE, Böger CA, Krämer BK, Doring A, Eheberg D, Fischer B, John J, Koenig W & Meisinger C: Effect of chronic kidney disease and comorbid conditions on health care costs: A 10-year observational study in a general population. Am J Nephrol 31: 222-229, 2010. 2. Wichmann HE, Gieger C & Illig T: KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen 67 Suppl 1: S26-30, 2005.

KORA-F4	Prospective population-based	1804	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> none.	Using stored urine samples, urinary albumin concentration was measured with a latex enhanced nephelometric assay (Siemens Healthcare Diagnostics) on a Dade Behring BN2 apparatus. Urinary creatinine concentration was measured using a kinetic Jaffe method in KORA F4.	1. Baumeister SE, Böger CA, Krämer BK, Doring A, Eheberg D, Fischer B, John J, Koenig W & Meisinger C: Effect of chronic kidney disease and comorbid conditions on health care costs: A 10-year observational study in a general population. <i>Am J Nephrol</i> 31: 222-229. 2. Wichmann HE, Gieger C & Illig T: KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. <i>Gesundheitswesen</i> 67 Suppl 1: S26-30, 2005.
LIFELINES	3-generations, population-based	8085	<b>Study exclusions or disease enrichment:</b> living outside the 3 Northern provinces of The Netherlands. <b>Exclusions:</b> none.	Urinary albumin and creatinine were measured using the Roche Modular.	Stolk RP, Rosmalen JGM, Postma DS, de Boer RA, Navis G, Slaets JPJ, Ormel J, and Wolffenbuttel BHR. Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. <i>Eur. J. Epidemiol.</i> , vol. 23, no. 1, pp. 67-74, Jan. 2008.
MESA	Community-based cohort study	2511	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> none.	Urine albumin and creatinine were measured at the Clinical Chemistry Laboratory at Fletcher Allen Health Care (Burlington, Vt). Urine albumin and creatinine were measured by nephelometry and the rate Jaffe reaction, respectively.	Bild DE et al. Multi-ethnic study of atherosclerosis: objectives and design. <i>Am J Epidemiol</i> 156, 871-81 (2002).

MICROS	Cross-sectional, population-based study using extended pedigrees	504	<b>Study exclusions or disease enrichment:</b> <18 years of age. <b>Exclusions:</b> samples with overall SNP call rate < 95%, showing excess of heterozygosity, or being classified as outliers by IBS clustering analysis were excluded prior to further analyses.	The urinary albumin-to-creatinine ratio was measured on a point-of-care diabetes management platform (Bayer DCA 2000+ analyzer).	1. Pattaro C, Marroni F, Riegler A, Mascalzoni D, Pichler I, Volpato CB, Dal Cero U, De Grandi A, Egger C, Eisele A, Fuchsberger C, Gögele M, Pedrotti S, Pinggera GK, Stefanov SA, Vogl FD, Wiedermann CJ, Meitinger T, Pramstaller PP. The genetic study of three population microisolates in South Tyrol (MICROS): study design and epidemiological perspectives. BMC Med Genet. 2007;8:29. 2. Marroni F, Grazio D, Pattaro C, Devoto M, Pramstaller P. Estimates of genetic and environmental contribution to 43 quantitative traits support sharing of a homogeneous environment in an isolated population from South Tyrol, Italy. Hum Hered. 2008;65(3):175-82.
PREVEND	Population-based	3634	<b>Study exclusions or disease enrichment:</b> aged between 28-75 yrs, enriched for microalbuminuria. <b>Exclusions:</b> none.	Urinary albumin was determined from fresh urine samples by nephelometry (BNII; Dade Behring Diagnostic, Marburg, Germany). Intra- and inter-assay coefficients of variation were 2.2 and 2.6%, respectively.	Hillege HL, Fidler V, Diercks GFH, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans ROB, Janssen WMT, Grobbee DE, and de Jong PE. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. Circulation, vol. 106, no. 14, pp. 1777–82, Oct. 2002.
SHIP	Prospective population-based	2655	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> sample call rate < 92%, duplicate samples (by IBS estimation), individuals with reported / genotyped gender mismatch.	Urinary albumin was measured from spot first morning void urine by nephelometry (BNII, Dade Behring Diagnostica, Marburg, Germany). Intra-assay and interassay coefficients of variation were 4.3% and 4.4%, respectively. Urinary creatinine concentration was measured using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY). Intra-assay and interassay coefficients of variation were 0.9% and 2.9%, respectively.	1. John U et al. Study of Health in Pomerania (SHIP). A health examination in an east German region: objectives and design. Soz Praventivmed 46:186-194, 2001. 2. Völzke H et al. Cohort Profile: The Study of Health in Pomerania. Int J Epidemiol, vol. 40, no. 2, pp. 294–307, Apr. 2011.

SHIP-TREND	Prospective population-based	985	<b>Study exclusions or disease enrichment:</b> this analysis concerns the subset of 988 individuals with genotype information. <b>Exclusions:</b> sample call rate < 94%, duplicate samples (by IBS estimation), individuals with reported/genotyped gender mismatch.	In a sample of spot urine, both the urinary albumin (intra-assay CV 4.5-7.6% for 1.0-24.5 mg/dl) and creatinine (Jaffe method, intra-assay CV 1.4-2.1% for 5.7-14.6 mmol/l) were measured on a Siemens Dimension Vista 1500 analyzer (Siemens Healthcare Diagnostics, Marburg, Germany), respectively.	1. John U et al. Study of Health in Pomerania (SHIP). A health examination in an east German region: objectives and design. <i>Soz Präventivmed</i> 46:186-194, 2001. 2. Völzke H et al. Cohort Profile: The Study of Health in Pomerania. <i>Int J Epidemiol</i> , vol. 40, no. 2, pp. 294–307, Apr. 2011.
<b>Replication study</b>					
ESTHER	Prospective study	2958	<b>Study exclusions or disease enrichment:</b> study participants were required to be ≥50 year old and having a good knowledge of the German language. <b>Exclusions:</b> samples with insufficient amount of DNA for genotyping.	Urinary albumin concentration was measured using nephelometric method (Siemens. Marburg, Germany). The urinary creatinine levels were photometrically measured using the modified kinetic Jaffe method (Greiner Diagnostic GmbH. Bahlingen, Germany).	1. Raum E, Rothenbacher D, Low M, Stegmaier C, Ziegler H, Brenner H. Changes of cardiovascular risk factors and their implications in subsequent birth cohorts of older adults in Germany: a life course approach. <i>Eur J Cardiovasc Prev Rehabil</i> 2007;14:809-814. 2. Schottker B, Haug U, Schomburg L, et al. Strong associations of 25-hydroxyvitamin D concentrations with all-cause, cardiovascular, cancer, and respiratory disease mortality in a large cohort study. <i>Am J Clin Nutr</i> 2013. 3. Weck MN, Stegmaier C, Rothenbacher D et al. Epidemiology of chronic atrophic gastritis: population-based study among 9444 older adults from Germany. <i>Aliment Pharmacol Ther.</i> 2007;26:879-887.
GANI_MED	Cohort study	1674	<b>Study exclusions or disease enrichment:</b> six main cohorts: heart failure, stroke, periodontal disease, renal insufficiency, metabolic syndrome, and fatty liver disease. <b>Exclusions:</b> sample call rate < 94%, heterozygosity rate > 6SD (MAF > 1%), PCA outliers (EV 1-4 > 8SD), duplicate samples (by IBS estimation), individuals with reported/genotyped gender mismatch..	In a sample of spot urine, the urinary albumin was measured on a Siemens Dimension Vista 1500 analyzer (Siemens Healthcare Diagnostics, Marburg, Germany). Urinary creatinine was measured either by an enzymatic or Jaffe method, whereas the analyses were adjusted accordingly for the method used.	Grabe HJ, Assel H, Bahls T et al. Cohort profile: Greifswald approach to individualized medicine (GANI_MED). <i>J. Transl. Med.</i> 2014; 12: 144.

GENDIAN	Cohort study	450	<b>Study exclusions or disease enrichment:</b> study on type 2 diabetes patients. <b>Exclusions:</b> of the 1,026 subjects undergoing genotyping, 53 were excluded due to call-rate < 95% (n=22), relatedness and duplicates (n=11), gender mismatch (n=16), ethnicity check (n=4); in addition, we excluded the following patients for the current analysis of cross-sectional UACR: patients with end-stage renal disease (n=438) or advanced, histologically proven diabetic nephropathy (n=84) or missing phenotype (n=1).	Urinary creatinine was measured using an enzymatic assay, urinary albumin was measured using the Roche Tina Quant assay.	1. Böger CA et al: effect of ACE and AT-2 inhibitors on mortality and progression to microalbuminuria in a nested case control study of diabetic nephropathy in diabetes mellitus type 2: results from the GENDIAN study. Int J Clin Pharmacol Ther 2006;44:364-74. 2. Böger CA et al. Association of eGFR-related loci identified by GWAS with incident CKD and ESRD. Plos Genet 2011;7:e1002292.
KORAF4 non-GWAS	Prospective population-based	1195	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> none.	Using stored urine samples, urinary albumin concentration was measured with a latex enhanced nephelometric assay (Siemens Healthcare Diagnostics) on a Dade Behring BN2 apparatus. Urinary creatinine concentration was measured using an enzymatic method.	1. Baumeister SE, Böger CA, Krämer BK, Doring A, Eheberg D, Fischer B, John J, Koenig W & Meisinger C: Effect of chronic kidney disease and comorbid conditions on health care costs: A 10-year observational study in a general population. Am J Nephrol 31: 222-229, 2010. 2. Wichmann HE, Gieger C & Illig T: KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen 67 Suppl 1: S26-30, 2005.
KORAF3 non-GWAS	Prospective population-based	1389	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> none.	Using stored urine samples, urinary albumin concentration was measured with a latex enhanced nephelometric assay (Siemens Healthcare Diagnostics) on a Dade Behring BN2 apparatus. Urinary creatinine concentration was measured using a kinetic Jaffe method in KORA F4.	1. Baumeister SE, Böger CA, Krämer BK, Doring A, Eheberg D, Fischer B, John J, Koenig W & Meisinger C: Effect of chronic kidney disease and comorbid conditions on health care costs: A 10-year observational study in a general population. Am J Nephrol 31: 222-229, 2010. 2. Wichmann HE, Gieger C & Illig T: KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen 67 Suppl 1: S26-30, 2005.



SAPHIR	Healthy working population	1690	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> none.	Urinary creatinine was measured using a modified kinetic Jaffe reaction (CREA, Roche Diagnostics GmbH, Mannheim, Germany). Urinary albumin concentration was determined using the Tinaquant assay (Roche Diagnostics GmbH, Mannheim, Germany).	1. Heid IM, Wagner SA, Gohlke H, Iglseder B, Mueller JC, Cip P, Ladurner G, Reiter R, Stadlmayr A, Mackevics V, Illig T, Kronenberg F, Paulweber B: Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes 55:375-384, 2006. 2. Kollerits B, Coassin S, Kiechl S, Hunt SC, Paulweber B, Willeit J, Brandstätter A, Lamina C, Adams TD, Kronenberg F: A common variant in the adiponutrin gene influences liver enzyme levels. Journal of Medical Genetics 47:116-119, 2010.
SKIPOGH	Cross-sectional family-based population-based	807	<b>Study exclusions or disease enrichment:</b> none. <b>Exclusions:</b> of the 941 participants who underwent genotyping, we excluded 71 participants with call rate < 90%, resulting in a total of 870 genotyped individuals.	Urinary creatinine was measured using an IDMS-traceable Jaffe kinetic compensated method. Urinary albumin concentration was measured using a quantitative immuno-nephelometry.	Pruijm M, Ponte B, Ackermann D, Vuistiner P, Paccaud F, Guessous I, Ehret G, Eisenberger U, Mohaupt M, Burnier M, Martin PY, Bochud M. Eur Radiol. 2013 May 28. [Epub ahead of print].
Vanderbilt Omni1	Practice-based cohort	472	<b>Study exclusions or disease enrichment:</b> samples chosen based on being a case or control for one of 31 pharmacogenetic analyses. <b>Exclusions:</b> individuals of non-white ancestry in the electronic medical record. Also excluded any lab measurements of individuals after initiation of dialysis or a kidney transplant.	The urinary albumin concentration was measured using turbidimetric immunoassay with endpoint determination. Urinary creatinine levels were measured using the modified Jaffé method.	
Vanderbilt Omni5	Practice-based cohort	144	<b>Study exclusions or disease enrichment:</b> samples chosen based on being a case or control for one of 31 pharmacogenetic analyses. <b>Exclusions:</b> individuals of non-white ancestry in the electronic medical record. Also excluded any lab measurements of individuals after initiation of dialysis or a kidney transplant.	The urinary albumin concentration was measured using turbidimetric immunoassay with endpoint determination. Urinary creatinine levels were measured using the modified Jaffé method.	

Vanderbilt 660W	Practice-based cohort	365	<b>Study exclusions or disease enrichment:</b> samples chosen for normal cardiac conduction, meaning that at some point in time they had a normal electrocardiogram without the presence of heart disease, arrhythmias, or electrocardiographically-active medications. <b>Exclusions:</b> children (age <18) and individuals of non-white ancestry in the electronic medical record. Also excluded any lab measurements from individuals after initiation of dialysis or a kidney transplant. At some point in their electronic medical record, the patients were absent of heart disease, but could later develop it.	The urinary albumin concentration was measured using turbidimetric immunoassay with endpoint determination. Urinary creatinine levels were measured using the modified Jaffé method.	Denny JC, Ritchie MD, Crawford DC, Schildcrout JS, Ramirez AH, Pulley JM, Basford MA, Masys DR, Haines JL, Roden DM. Identification of genomic predictors of atrioventricular conduction: Using electronic medical records as a tool for genome science. Circulation 2010;122(20):2016-21.
<b>Clinical characterization study</b>					
DCCT/EDIC	Trial of patients with type I diabetes	1304	<b>Study exclusions or disease enrichment:</b> individuals with insulin-dependent type I diabetes mellitus between 1 and 15 years of duration, age 13-39 years at enrolment, free of advanced diabetes-related complications, absence of several comorbidities. <b>Exclusions:</b> Subjects meeting the criteria for persistent microalbuminuria at DCCT baseline and DCCT year 1 (n = 60) were excluded from the analyses of the time to incident albuminuria. Analyses were restricted to individuals of European ancestry.	The urinary albumin concentration was measured from times urine samples using a solid-phase fluoroimmunoassay. Urinary creatinine levels were measured using the Jaffé method.	1. The Diabetes Control and Complications (DCCT) Research Group. Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. Kidney Int 1995;47(6):1703–20. 2. de Boer IH et al. Long-term renal outcomes of patients with type 1 diabetes mellitus and microalbuminuria: an analysis of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort. Arch Intern Med. 2011 Mar 14;171(5):412-20.

Supplementary Table 3: Study-specific information about genotyping, imputation and data management and analysis

Study Name	Genotyping Array type	Genotype calling algorithm	QC filters for genotyped SNPs used for imputation (listed are criteria for exclusion)	No of SNPs used for imputation	Imputation software, version	Imputation Backbone (NCBI build)	Filtering of imputed genotypes	Data management and statistical analysis
<b>3C</b>	Illumina Human610-Quad	BeadStudio	call rate < 98%, pHWE < 10E-6, MAF < 1%	492,897	MACH	1000 Genomes EUR, Dec 2010 (Build 37)	none	R and ProbABEL
<b>Advance</b>	Affymetrix 5.0 Affymetrix 6.0	Affymetrix	SNPs genotyped on Affymetrix 5.0: call rate < 96% (<99% if MAF < 5%); SNPs genotyped on Affymetrix 6.0: call rate < 97% (<99% if MAF < 5%)	876,688	IMPUTE2 2.1.2	1000 Genomes CEU Pilot, Jun 2010 plus HapMap 3 rel. 2 all available haplotypes, Feb 2009 (build 36)	imputation info < 0.5	SNPTEST
<b>AGES</b>	Illumina Hu370CNV	Illumina	call rate < 97%, pHWE < 1e-6, MAF < 0.01, mishap p < 1e-9, SNPs not in Hapmap or strandedness issues merging with Hapmap	329,804	MACH 1.0.16	HapMap rel. 22 (build 36)	none	R, ProbABEL, Linear and Logistic Regression
<b>Amish</b>	Affymetrix 500K	BRLMM	call rate < 95%, pHWE < 10E-6, MAF < 1%, non-HapMap	338,598	MACH 1.0.15	HapMap rel. 22 phased CEU haplotypes (build 36)	none	Measured genotype accounting for polygenic component
<b>ARIC</b>	Affymetrix 6.0	Birdseed	call rate < 95%, pHWE < 10E-5, MAF < 1%	669,450	MACH 1.0.16	HapMap rel. 22 (build 36)	none	ProbABEL, PLINK, R
<b>BLSA</b>	Illumina Infinium HumanHap 550K	Beadstudio	call rate < 99%, pHWE < 10E-4, MAF < 1%	501,764	MACH 1.0.15	HapMap rel. 21 phased CEU haplotypes (build 35)	MAF < 1%, r2hat < 0.3	SAS, Merlin, R
<b>CHS</b>	Illumina 370CNV	BeadStudio	call rate < 97%, pHWE < 10E-5, heterozygotes=0, SNP not in HapMap	306,655	BimBam 0.99	HapMap rel. 22 (build 36)	dosage variance < 0.01	Linear and logistic regression using R, robust estimates of SE
<b>COLAUS</b>	Affymetrix 500K	BRLMM	call rate < 70%, pHWE < 10E-7	390,631	IMPUTE 0.2.0	HapMap rel. 21 (build 35)	none	Matlab
<b>CROATIA-SPLIT</b>	HAP370CNV	Illumina	call rate < 98%, pHWE < 10E-10	330,997	MACH 1.0.15	HapMap rel. 22 CEU haplotypes (build 36)	none	R (GenABEL, ProABEL)
<b>EPIC</b>	Affymetrix 500K	BRLMM	call rate < 90%, pHWE < 10e-6	382,037	IMPUTE 0.3.1	HapMap rel. 21 (Build 35)	none	SAS, Stata, Linux scripts
<b>Fenland</b>	Affymetrix 500K	BRLMM	call rate < 90%, pHWE < 10E-6, MAF < 1%	362,055	IMPUTE 0.4.2	HapMap rel. 22 (build 36)	proper_info < 0.4	Linux, Stata 10.1, SNPTEST 1.1.5
<b>FHS</b>	Affymetrix 500K Affymetrix 50K	Affymetrix	call rate < 95%, pHWE < 10E-6	503,526	MACH 1.0.15	HapMap rel. 22 phased CEU haplotypes (build 36)	none	R

	supplemental							
INCIPE	Illumina	Illumina	call rate < 95%, pHWE < 10E-6	635,646	IMPUTE 0.2.0	HapMap rel. 22 phased CEU haplotypes (build 36)	none	R
KORA-F3	Affymetrix 500K	BRLMM	per-chip call rate < 93%, MAF < 5%, discrepancy for one of the 50 SNPs common on both chips, gender checks	380,407	MACH	HapMap rel. 22 (build 35)	none	MACH2QTL, ProbABEL, R, Visual Basic
KORA-F4	Affymetrix 6.0	BRLMM	per-chip call rate < 93%, per SNP call rate < 93%, MAF < 1%, gender checks	629,893	MACH	HapMap rel. 22 (build 36)	none	MACH2QTL, ProbABEL, R, Visual Basic
LIFELINES	Illumina CytoSNP12 v2	GenomeStudio	call rate < 95%, pHWE < 1E-05	257,581		HapMap rel. 22 phased CEU haplotypes (build 36)	none	NO
MESA	Affymetrix Genome-Wide Human SNP Array 6.0	Birdseed v2	call rate < 95%, MAF ≤ 1%	897,979	IMPUTE 2.1.0	HapMap rel. 22 phased CEU haplotypes (build 36)	none	PLINK
MICROS	Illumina Infinium HumanHap300 v2 SNP bead microarrays	Beadstudio	call rate < 98%, pHWE < 10E-6, MAF < 1%	292,917	MACH 1.0.16	HapMap rel. 22 (build 36)	none	R, GenABEL, ProbABEL;
PREVEND	Illumina CytoSNP12 v2	GenomeStudio	call rate < 95%, pHWE < 1E-05	232,571		HapMap rel. 22 phased CEU haplotypes (build 36)	none	NO
SHIP	Affymetrix 6.0	Birdseed2	none	869,224	IMPUTE 0.5.0	HapMap rel. 22 (build 36)	none	SNPTEST 1.1.5, QUICKTEST 0.94, R, InforSense, InterSystems Caché
SHIP-TREND	Illumina Human Omni 2.5	GenomeStudio	call rate ≤ 0.9, pHWE ≤ 1E-04, monomorphic SNPs	1,782,967	IMPUTE 2.1.2.3	HapMap rel. 22 phased CEU haplotypes (build 36)	duplicate RSID but different positions	QUICKTEST 0.95, R, InforSense, InterSystems Caché
<i>in silico replication</i>								
GANI_MED	Illumina Infinium PsychArray	GenomeStudio	call rate ≤ 0.95, pHWE ≤ 1E-04, MAF ≤ 0.005	305,145	IMPUTE 2.3.1	1000 Genomes Phase I v3 ALL (macGT1) (build 37)	duplicate IDs (via positions)	R, PLINK, gtool, InterSystems Caché
GENDIAN	Genome-Wide Human SNP Array 6.0	Birdseed (BRLMM)	n=126,259 SNPs (chr 1-chr22, chr X) were excluded from imputation by SNP QC due to one of the following: HWE-p < 10-6; monomorphic SNPs; MAF>.1 & call rate<.9 MAF>.09 & MAF <=.1 & call rate<.91 MAF>.08 & MAF <=.09 & call rate<.92	747,402	MACH 1.0.18.c MiniMac 2012-10-09	GIANT ALL 1000G v3 ref panel GRCh (build 37)	none	R

			MAF>.07 & MAF <=.08 & call rate<.93 MAF>.06 & MAF <=.07 & call rate<.94 MAF>.05 & MAF <=.06 & call rate<.95 MAF>.04 & MAF <=.05 & call rate<.96 MAF>.03 & MAF <=.04 & call rate<.97 MAF>.02 & MAF <=.03 & call rate<.98 MAF>.01 & MAF <=.02 & call rate<.99 MAF <=.01 & call rate<.99					
<b>Vanderbilt Omni1</b>	Illumina HumanOmni1-Quad	BeadStudio	call rate < 98%, IBD (Z0<0.8), Mendel errors > 0, Duplicate concordance < 100%	946,523	IMPUTE 2.3.0	1000 Genomes Phase 1 integrated v3	Genotype Likelihood <0.9	Plink and R
<b>Vanderbilt Omni5</b>	Illumina HumanOmni5-Quad	BeadStudio	call rate < 98%, IBD (Z0<0.8), Mendel errors > 0, Duplicate concordance < 100%	3,819,154	IMPUTE 2.3.0	1000 Genomes Phase 1 integrated v3	Genotype Likelihood <0.9	Plink and R
<b>Vanderbilt 660W</b>	Illumina Human660W-Quad	BeadStudio	call rate < 98%, IBD (Z0<0.8), Mendel errors > 0, Duplicate concordance < 100%	530,014	IMPUTE 2.3.0	1000 Genomes Phase 1 integrated v3	Genotype Likelihood <0.9	Plink and R
<b>de novo replication</b>								
	<b>genotyping platform</b>	<b>amount of DNA used per SNP (in ng)</b>	<b>genotyping method</b>	<b>n duplicates and concordance per SNP (provide per individual SNP)</b>	<b>number attempted /number genotyped (per individual SNP)</b>	<b>Other QC indices that your lab uses</b>		
<b>ESTHER</b>	LGC genomics SNP-line, using KASP Chemistry and 1536-well plates	3.75	De novo genotyping using KASPar v4.0 after whole genome amplification by primer extension preamplification (PEP) using thermostable DNA polymerases	LGC Genomics does not add duplicates. The data for each SNP represents one reaction per sample.	call rate range 0.98 - 1	none indicated by the lab		
<b>SKIPOGH</b>	LGC genomics SNP-line, using KASP Chemistry and 1536-well plates	5 -7.5	De novo genotyping using KASPar v4.0 after whole genome amplification by primer extension preamplification (PEP) using thermostable DNA polymerases	29 participants were genotyped in duplicate. SNP concordance	SNP call rates varied from 94.5% to 99.5% (median 97.2)	All assays have been validated on an in-house DNA panel (44 random Caucasian DNA samples). All sample plates genotyped include at least two negative controls. ie.		

				varied between 86% and 100%.		blank/water controls. All genotyping data are initially generated by an automated algorithm (genotype calling based upon recorded fluorescence values). All genotyping data is manually checked and verified by no less than two experienced scientists at LGC genomics.		
KORAF4 non-GWAS	Mass ARRAY Analyzer 4 system	15	iPlex Gold	At least 15% duplicate genotyping per SNP. Concordance ≥95%, median = 100%	NA	NA		
KORAF3 non-GWAS	Mass ARRAY Analyzer 4 system	15	iPlex Gold	At least 15% duplicate genotyping per SNP. Concordance ≥95%, median = 100%	NA	NA		
SAPHIR	Mass ARRAY Analyzer 4 system	15	iPlex Gold	70 duplicates; 46 SNPs were genotyped; 44 SNPs had a concordance of 100%; 2 SNPs had each 1 discordant sample	46 SNPs were genotyped and had an average call rate of 99,3% (between 98.15% and 99.65%)	automatic calculation of the HWE, comparison of the obtained genotypes with HapMap Data		

Supplementary Table 4: SNPs associated with UACR among all individuals with a p-value of &lt;1E-05.

SNPID	chr	position (hg18)	Allele1	Allele2	Fre- quency Allele1	Effect	SE	p-value	I <sup>2</sup> %	Sample Size	In Gene	Genes Within 100kb
rs880315	1	10719453	t	c	0.65	-0.042	0.009	9.1E-06	0	41333	CASZ1	MIR92B(dist=2901),THBS3(dist=3312),TRIM46(dist=4620),KRTCA P2(dist=16263),MTX1(dist=16423),GBAP1(dist=21549),GBA(dist= 42172),DPM3(dist=49071),SLC50A1(dist=50733),EFNA1(dist=546 81),FAM189B(dist=54929),SCAMP3(dist=63703),CLK2(dist=70592 ) ,HCN3(dist=85151),PKLR(dist=97017)
rs4072037	1	153428691	t	c	0.54	0.029	0.006	2.5E-06	0	54450	MUC1	MTX1(dist=2598),GBAP1(dist=7724),MIR92B(dist=10829),MUC1( dist=13186),TRIM46(dist=18445),GBA(dist=28347),KRTCAP2(dist= 30088),FAM189B(dist=41104),SCAMP3(dist=49878),CLK2(dist=56 767),DPM3(dist=62896),SLC50A1(dist=64558),EFNA1(dist=68506) ) ,HCN3(dist=71326),PKLR(dist=83192)
rs914615	1	153442516	a	g	0.47	-0.030	0.007	7.4E-06	0	44877	THBS3	
rs17346504	2	137640231	t	c	0.12	0.050	0.011	7.2E-06	27	53401	THSD7B	
rs9333289	2	187206352	t	c	0.70	-0.030	0.007	9.3E-06	24	54441	ITGAV	FAM171B(dist=60682)
rs9333290	2	187227583	t	g	0.30	0.038	0.008	7.5E-07	15	54441	ITGAV	FAM171B(dist=39451)
rs13006483	2	187230995	t	g	0.30	0.037	0.008	1.2E-06	15	54441	ITGAV	FAM171B(dist=36039)
rs3816386	2	187236880	a	g	0.69	-0.035	0.007	2.9E-06	0	54441	ITGAV	FAM171B(dist=30154)
rs11685758	2	187241613	t	c	0.31	0.039	0.008	2.7E-06	0	44877	ITGAV	FAM171B(dist=25421)
rs12151442	2	187246092	t	c	0.70	-0.030	0.007	5.5E-06	1	54441	ITGAV	FAM171B(dist=20942)
rs13001028	2	187255140	a	g	0.69	-0.035	0.007	2.0E-06	0	54440		ITGAV(dist=1266),FAM171B(dist=11894)
rs13028817	2	187255744	t	g	0.70	-0.029	0.007	7.3E-06	0	54439		ITGAV(dist=1870),FAM171B(dist=11290)
rs12615659	2	187259552	a	t	0.30	0.030	0.007	4.3E-06	2	54439		ITGAV(dist=5678),FAM171B(dist=7482)
rs11678190	2	187268553	a	c	0.69	-0.036	0.007	1.5E-06	0	54441	FAM171B	ITGAV(dist=14679)
rs17750683	2	187328542	a	t	0.68	-0.033	0.007	4.1E-06	22	54439	FAM171B	ZSWIM2(dist=71910),ITGAV(dist=74668)
rs13026081	2	187334583	t	c	0.32	0.032	0.007	6.8E-06	21	54434	FAM171B	ZSWIM2(dist=65869),ITGAV(dist=80709)
rs11783652	8	55021047	a	g	0.32	0.037	0.008	2.4E-06	0	54450	RGS20	TCEA1(dist=20620)
rs17301329	8	55021534	a	t	0.29	0.042	0.008	5.6E-07	0	54450	RGS20	TCEA1(dist=20133),LYPLA1(dist=99946)
rs16919699	8	55021582	t	c	0.66	-0.037	0.008	2.3E-06	0	54450	RGS20	TCEA1(dist=20085),LYPLA1(dist=99898)
rs1016013	9	96516305	a	g	0.42	0.028	0.006	6.4E-06	5	54450		C9orf3(dist=12467),FBP1(dist=73953),MIR2278(dist=95760)
rs7851726	9	96543806	t	c	0.42	0.027	0.006	5.2E-06	3	54450	C9orf3	MIR2278(dist=68259)
rs446540	9	96549020	a	g	0.43	0.028	0.006	5.8E-06	10	54304	C9orf3	MIR2278(dist=63045)
rs183066	9	96557253	t	c	0.57	-0.028	0.006	5.8E-06	9	54448	C9orf3	MIR2278(dist=54812)
rs2584806	9	96569099	a	c	0.58	-0.027	0.006	9.3E-06	9	54449	C9orf3	MIR2278(dist=42966)
rs1109861	10	11286275	a	c	0.55	-0.030	0.006	1.9E-06	5	54442	CELF2	CELF2-AS2(dist=98818)
rs1801239	10	16959058	t	c	0.90	-0.066	0.011	4.6E-09	31	54450	CUBN	RSU1(dist=59599)

Diabetes

rs17343073	10	16972202	a	t	0.90	-0.071	0.012	4.0E-09	22	54449	CUBN	RSU1(dist=72743)
rs6602163	10	17006772	a	g	0.84	-0.056	0.009	1.2E-09	5	54450	CUBN	
rs10795433*	10	17009929	a	c	0.86	-0.061	0.010	2.4E-10	6	54450	CUBN	
rs2417849	12	20167780	t	c	0.37	0.028	0.006	9.5E-06	39	54441		LOC100506393(dist=24711)
rs2303658	12	20169697	a	g	0.34	0.030	0.007	9.5E-06	31	54442		LOC100506393(dist=26628)
rs11609944	12	20170557	a	g	0.38	0.028	0.006	9.6E-06	42	54449		LOC100506393(dist=27488)
rs1728897	15	53088662	t	c	0.54	-0.028	0.006	4.1E-06	0	54433		
rs12594729	15	53088684	a	g	0.50	0.029	0.006	2.0E-06	0	54450		
rs7167661	15	53090751	t	c	0.54	-0.028	0.006	3.5E-06	0	54450		
rs11071163	15	53091242	a	g	0.50	-0.029	0.006	9.2E-06	0	54449		
rs7173577	15	53092295	a	g	0.45	-0.029	0.006	2.3E-06	0	54450		
rs1728867	15	53094106	a	g	0.45	-0.030	0.006	8.3E-07	0	54449		
rs951048	15	53094503	a	t	0.44	-0.030	0.006	8.7E-07	0	54449		
rs2414396	15	53094680	a	g	0.46	-0.031	0.006	7.6E-07	0	54449		
rs12907410	15	53095223	t	c	0.56	0.028	0.006	3.7E-06	0	54449		
rs1728886	15	53095714	t	c	0.56	0.030	0.006	1.2E-06	0	54449		
rs17818939	15	53096140	a	g	0.44	-0.030	0.006	1.1E-06	0	54450		
rs1728878	15	53097144	t	c	0.57	0.028	0.006	1.9E-06	0	54450		
rs8042768	15	53097375	a	g	0.43	-0.028	0.006	2.1E-06	0	54448		
rs1690363	15	53098119	a	g	0.43	-0.028	0.006	2.0E-06	0	54448		
rs1690365	15	53098549	t	c	0.56	0.028	0.006	1.9E-06	0	54450		
rs1614271	15	53098677	t	c	0.57	0.029	0.006	1.6E-06	0	54448		
rs1690366	15	53098855	t	g	0.44	-0.030	0.006	2.0E-06	0	54448		
rs1690367	15	53099066	a	g	0.43	-0.028	0.006	1.8E-06	0	54406		
rs7180127	15	53103432	t	c	0.51	0.029	0.006	3.7E-06	0	54449		
rs10083619	15	53106962	a	g	0.51	0.029	0.006	3.5E-06	0	54448		
rs2899576	15	53107909	t	c	0.48	-0.030	0.006	1.2E-06	0	54424		
rs1528472	15	53108420	a	c	0.48	-0.032	0.006	5.4E-07	0	54445		
rs17238122	15	53109188	a	g	0.48	-0.031	0.006	8.8E-07	0	54443		
rs1528477	15	53111680	a	g	0.48	-0.031	0.006	1.5E-06	0	54449		
rs1830324	15	53112207	a	g	0.51	-0.030	0.006	3.4E-06	0	54449		
rs11858741	15	53112699	a	g	0.51	0.030	0.006	2.2E-06	0	54450		
rs231226	19	40959617	t	c	0.62	-0.033	0.007	5.1E-06	22	44877	ARHGAP33	PROSER3(dist=7700),LINC01529(dist=12001),HSPB6(dist=19847),LIN37(dist=22357),PRODH2(dist=23115),PSENEN(dist=29721),U2AF1L4(dist=31434),IGFLR1(dist=34426),KMT2B(dist=37996),NPHS1(dist=48497),ZBTB32(dist=59837),KIRREL2(dist=80033),APLP1(dist=91624),UPK1A(dist=98390)
rs231227	19	40959907	a	g	0.38	0.033	0.007	4.9E-06	22	44877	ARHGAP33	PROSER3(dist=7990),LINC01529(dist=11711),HSPB6(dist=20137),LIN37(dist=22647),PRODH2(dist=22825),PSENEN(dist=30011),U2AF1L4(dist=31724),IGFLR1(dist=34716),KMT2B(dist=38286),NPHS1(dist=48207),ZBTB32(dist=60127),KIRREL2(dist=79743),APLP1(dist=91334),UPK1A(dist=98680)



rs2828785	21	24359376	t	c	0.27	-0.038	0.008	7.9E-06	0	54450
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Standard error (SE) and p-values are corrected for genomic control. A1 is the coded allele.

\*The previously identified missense variant rs18012399 in *CUBN* is correlated with the index variant rs10795433 in this study ( $r^2=0.54$  and  $D'=1$ , based on HapMap r22 CEU data)

Supplementary Table 5: SNPs associated with MA among all individuals with a p-value of <1E-05.

SNPID	chr	position (hg18)	Allele 1	Allele 2	Fre- quency Allele1	Effect	SE	p-value	I <sup>2</sup> %	Sample Size	In Gene	Genes Within 100kb
rs11579312	1	30429159	t	c	0.69	0.11	0.025	9.7E-06	0	54116		
rs3795324	1	158909735	a	c	0.82	-0.15	0.031	9.4E-07	22	52716		CD48(dist=5425),SLAMF1(dist=26010),SLAMF7(dist=65736),CD84(dist=93805)
rs16827742	2	150615405	a	g	0.06	0.30	0.063	3.1E-06	12	35962		
rs9333289	2	187206352	t	c	0.71	-0.10	0.022	5.2E-06	0	54107	ITGAV	FAM171B(dist=60682)
rs9333290	2	187227583	t	g	0.29	0.11	0.023	5.0E-06	0	54107	ITGAV	FAM171B(dist=39451)
rs13006483	2	187230995	t	g	0.29	0.10	0.023	7.0E-06	0	54107	ITGAV	FAM171B(dist=36039)
rs12151442	2	187246092	t	c	0.70	-0.10	0.022	2.0E-06	0	54107	ITGAV	FAM171B(dist=20942)
rs13001028	2	187255140	a	g	0.70	-0.10	0.023	8.3E-06	0	54106		ITGAV(dist=1266),FAM171B(dist=11894)
rs13028817	2	187255744	t	g	0.70	-0.10	0.022	2.1E-06	0	54105		ITGAV(dist=1870),FAM171B(dist=11290)
rs12615659	2	187259552	a	t	0.30	0.11	0.022	1.3E-06	0	54105		ITGAV(dist=5678),FAM171B(dist=7482)
rs11678190	2	187268553	a	c	0.70	-0.10	0.023	5.1E-06	0	54107	FAM171B	ITGAV(dist=14679)
rs17750683	2	187328542	a	t	0.68	-0.11	0.022	1.4E-06	0	54105	FAM171B	ZSWIM2(dist=71910),ITGAV(dist=74668)
rs13026081	2	187334583	t	c	0.32	0.11	0.022	1.6E-06	0	54093	FAM171B	ZSWIM2(dist=65869),ITGAV(dist=80709)
rs1077216	3	46867165	t	c	0.07	0.20	0.044	5.2E-06	5	45096		MYL3(dist=7196),PRSS42(dist=16576),PTH1R(dist=27075),CCDC12(dist=71059)
rs13160548	5	38814607	t	c	0.69	-0.10	0.023	8.2E-06	14	53130	OSMR-AS1	LINC01265(dist=58475),OSMR(dist=67110)
rs12719264	5	119211839	a	g	0.30	-0.11	0.025	6.2E-06	29	54115		
rs2110904	6	107701464	t	c	0.65	0.10	0.022	8.9E-06	0	54116	PDSS2	
rs538641	8	103072879	a	g	0.05	0.28	0.062	7.8E-06	0	50048	NCALD	
rs1801239	10	16959058	t	c	0.90	-0.23	0.035	1.7E-10	18	54115	CUBN	RSU1(dist=59599)
rs17343073	10	16972202	a	t	0.90	-0.23	0.036	3.0E-10	0	54115	CUBN	RSU1(dist=72743)
rs6602163	10	17006772	a	g	0.83	-0.17	0.029	1.5E-09	5	54116	CUBN	
rs10795433	10	17009929	a	c	0.85	-0.20	0.031	1.3E-10	4	54116	CUBN	
rs12764441	10	72361657	t	c	0.48	-0.10	0.021	3.5E-06	0	54116		PCBD1(dist=43108),SGPL1(dist=50719)
rs3740393	10	104626645	c	g	0.21	0.13	0.028	6.1E-06	19	54048	C10orf32-ASMT	C10orf32(dist=11937),CYP17A1(dist=39365),CNNM2(dist=41420),WBP1L(dist=60634)
rs10899033	11	74070819	c	g	0.72	0.11	0.025	9.3E-06	0	54116		CHRD12(dist=14303),MIR4696(dist=38142),POLD3(dist=39066),RNF169(dist=66742)
rs10498273	14	20214639	c	g	0.94	-0.21	0.047	9.6E-06	36	53131		ANG(dist=7537),RNASE4(dist=7573),OR6S1(dist=34949),EDDM3A(dist=69300),LOC254028(dist=69419),RNASE12(dist=85817),RNASE11(dist=86382),EDDM3B(dist=91787)
rs7145202	14	22161945	t	c	0.62	0.10	0.022	3.7E-06	0	54106		ABHD4(dist=10840),DAD1(dist=33962)
rs6572602	14	22163380	a	g	0.62	0.11	0.024	4.6E-06	0	41412		ABHD4(dist=12275),DAD1(dist=35397)
rs274173	19	61384255	c	g	0.17	-0.23	0.051	5.2E-06	12	38796	GALP	ZSCAN5B(dist=8615),ZNF444(dist=20181),ZSCAN5A(dist=40236),ZNF787(dist=59701)

rs6030216	20	40486448	t	c	0.17	0.12	0.027	6.0E-06	0	54115	PTPRT
rs4812598	20	40487956	c	g	0.83	-0.12	0.027	9.1E-06	0	54115	PTPRT
rs6513791	20	40491536	t	c	0.18	0.12	0.026	4.4E-06	12	54115	PTPRT
rs4810356	20	40491604	t	c	0.82	-0.13	0.028	7.6E-06	11	54115	PTPRT
rs6030232	20	40496297	a	t	0.82	-0.12	0.027	8.7E-06	0	54115	PTPRT
rs6030238	20	40498930	a	g	0.81	-0.12	0.026	6.0E-06	12	54115	PTPRT

Odds ratios can be obtained by exponentiating the effect to the basis  $e$ .

Supplementary Table 6: SNPs associated with UACR among individuals without diabetes with a p-value of <1E-05.

SNPID	chr	position (hg18)	Allele 1	Allele 2	Fre- quency Allele1	Effect	SE	p-value	I <sup>2</sup> %	Sample Size	In Gene	Genes Within 100kb
rs17377079	1	84999401	a	g	0.15	0.060	0.013	6.9E-06	9	46061		LPAR3(dist=52273),SSX2IP(dist=70573)
rs4072037	1	153428691	t	c	0.54	0.028	0.006	8.5E-06	0	46061	MUC1	MIR92B(dist=2901),THBS3(dist=3312),TRIM46(dist=4620),KRTC AP2(dist=16263),MTX1(dist=16423),GBAP1(dist=21549),GBA(di st=42172),DPM3(dist=49071),SLC50A1(dist=50733),EFNA1(dist =54681),FAM189B(dist=54929),SCAMP3(dist=63703),CLK2(dist =70592),HCN3(dist=85151),PKLR(dist=97017)
rs9333290	2	187227583	t	g	0.30	0.037	0.008	4.1E-06	3	46052	ITGAV	FAM171B(dist=39451)
rs13006483	2	187230995	t	g	0.30	0.035	0.008	6.7E-06	3	46052	ITGAV	FAM171B(dist=36039)
rs13001028	2	187255140	a	g	0.69	-0.034	0.008	9.9E-06	0	46052		ITGAV(dist=1266),FAM171B(dist=11894)
rs11678190	2	187268553	a	c	0.69	-0.035	0.008	8.7E-06	0	46052	FAM171B	ITGAV(dist=14679)
rs17750683	2	187328542	a	t	0.68	-0.035	0.008	4.6E-06	0	46052	FAM171B	ZSWIM2(dist=71910),ITGAV(dist=74668)
rs13026081	2	187334583	t	c	0.32	0.034	0.008	8.3E-06	0	46045	FAM171B	ZSWIM2(dist=65869),ITGAV(dist=80709)
rs4674086	2	201032130	t	c	0.46	0.028	0.006	8.7E-06	0	45053	SPATS2L	KCTD18(dist=29799),SGOL2(dist=66980)
rs9372871	6	127849645	t	c	0.89	-0.046	0.010	4.2E-06	2	45094	SOGA3	KIAA0408(dist=27417),C6orf58(dist=90367)
rs9372872	6	127849848	c	g	0.11	0.046	0.010	2.5E-06	0	46061	SOGA3	KIAA0408(dist=27620),C6orf58(dist=90164)
rs7739650	6	127850605	a	g	0.11	0.046	0.010	3.1E-06	2	46061	SOGA3	KIAA0408(dist=28377),C6orf58(dist=89407)
rs13220247	6	127850652	t	c	0.89	-0.046	0.010	3.4E-06	2	46061	SOGA3	KIAA0408(dist=28424),C6orf58(dist=89360)
rs9388580	6	127851073	t	c	0.89	-0.044	0.010	8.7E-06	6	46061	SOGA3	KIAA0408(dist=28845),C6orf58(dist=88939)
rs12668467	7	13598753	t	c	0.27	-0.043	0.009	4.1E-06	0	46061		
rs1801239	10	16959058	t	c	0.90	-0.054	0.012	4.4E-06	25	46061	CUBN	RSU1(dist=59599)
rs10795433	10	17009929	a	c	0.86	-0.045	0.010	8.7E-06	14	46061	CUBN	
rs2192224	15	24959369	t	g	0.13	0.048	0.011	6.1E-06	0	46061	GABRG3	LOC101928869(dist=26259)
rs7173577	15	53092295	a	g	0.45	-0.029	0.006	6.7E-06	0	46061		
rs1728867	15	53094106	a	g	0.45	-0.028	0.006	7.4E-06	0	46061		
rs951048	15	53094503	a	t	0.44	-0.028	0.006	7.8E-06	0	46061		
rs2414396	15	53094680	a	g	0.46	-0.029	0.006	4.1E-06	0	46061		
rs17818939	15	53096140	a	g	0.44	-0.028	0.006	9.9E-06	0	46061		
rs2899576	15	53107909	t	c	0.48	-0.029	0.006	5.7E-06	0	46035		
rs1528472	15	53108420	a	c	0.48	-0.030	0.007	3.1E-06	0	46056		
rs17238122	15	53109188	a	g	0.48	-0.030	0.007	4.8E-06	0	46054		
rs1528477	15	53111680	a	g	0.48	-0.030	0.007	6.6E-06	0	46061		
rs11858741	15	53112699	a	g	0.51	0.029	0.007	7.9E-06	0	46061		
rs4528660	18	3033516	t	c	0.91	-0.073	0.017	9.4E-06	3	33478		MYOM1(dist=23289),LPIN2(dist=31571),LOC727896 (dist=96895)

Supplementary Table 7: SNPs associated with UACR among individuals with diabetes with a p-value of &lt;1E-05.

SNPID	chr	position (hg18)	Allele 1	Allele 2	Frequency Allele1	Effect	SE	p-value	I <sup>2</sup> %	Sample Size	In Gene	Genes Within 100kb [Closest Gene]
rs13427836	2	128744431	t	c	0.14	0.199	0.044	6.1E-06	10	5509	<i>HS6ST1</i>	UGGT1(dist=74712)
rs13428208	2	128744772	t	c	0.14	0.195	0.044	7.6E-06	10	5509	<i>HS6ST1</i>	UGGT1(dist=75053)
rs2405747	2	128748295	t	c	0.15	0.193	0.043	6.9E-06	14	5509	<i>HS6ST1</i>	UGGT1(dist=78576)
rs4662787	2	128752447	t	c	0.18	0.176	0.040	9.0E-06	0	5824	<i>HS6ST1</i>	UGGT1(dist=82728)
rs10183821	2	128753139	a	g	0.81	-0.169	0.038	9.3E-06	0	5825	<i>HS6ST1</i>	UGGT1(dist=83420)
rs13079877	3	2102845	a	g	0.45	0.148	0.033	5.6E-06	25	5825		CNTN4(dist=12705),CNTN4-AS2(dist=24248)
rs7634770	3	67012918	a	c	0.70	-0.142	0.030	2.7E-06	19	5825		[KBTBD8, dist=119174]
rs9876318	3	67014118	a	t	0.69	-0.144	0.030	2.0E-06	20	5824		[KBTBD8, dist=117974]
rs17738155	6	51264035	t	c	0.92	-0.241	0.053	5.9E-06	39	5825		[PKHD1, dist=324068]
rs947724	6	51274689	t	c	0.92	-0.239	0.053	7.5E-06	41	5825		[PKHD1, dist=313414]
rs7792461	7	29479920	t	g	0.39	0.130	0.029	5.1E-06	0	5825	<i>CHN2</i>	PRR15(dist=90032)
rs4722909	7	29481456	a	g	0.60	-0.134	0.029	3.2E-06	0	5823	<i>CHN2</i>	PRR15(dist=88496)
rs4722913	7	29482735	a	g	0.61	-0.131	0.029	4.2E-06	0	5825	<i>CHN2</i>	PRR15(dist=87217)
rs7798161	7	29483162	a	g	0.61	-0.130	0.029	4.7E-06	0	5825	<i>CHN2</i>	PRR15(dist=86790)
rs3828977	7	29486023	a	g	0.59	-0.131	0.029	4.9E-06	0	5825	<i>CHN2</i>	PRR15(dist=83929)
rs7922045	10	122991722	t	c	0.26	0.165	0.033	5.7E-07	0	5824		[FGFR2, dist=236111]
rs729014	10	122992796	t	c	0.15	0.202	0.043	2.4E-06	0	5825		[FGFR2, dist=235037]
rs649529	11	87647899	t	g	0.43	-0.147	0.033	9.3E-06	0	5825		CTSC(dist=18509),RAB38(dist=99616)

Supplementary Table 8: Discovery, replication and combined estimates for all index SNPs associated with UACR in diabetes in the discovery sample at p<1E-05

Marker	gene nearby	chr	position (hg18)	A A		discovery						replication						combined					
				1	2	Freq A1	beta	SE	p-value	I <sup>2</sup> %	n	Freq A1	beta	SE	p-value	I <sup>2</sup> %	n	Freq A1	beta	SE	p-value	I <sup>2</sup> %	n
rs13427836	HS6ST1	2	128744431	t	c	0.14	0.20	0.04	6.1E-06	10	5509	0.15	0.16	0.07	3.13E-02	58	1890	0.15	0.19	0.04	6.31E-07	30	7399
rs13079877	CNTN4	3	2102845	a	g	0.45	0.15	0.03	5.6E-06	25	5825	0.50	0.04	0.05	5.16E-01	0	1880	0.46	0.12	0.03	2.40E-05	20	7705
rs9876318	KBTD8	3	67014118	a	t	0.69	-0.14	0.03	2.0E-06	20	5824	0.69	0.08	0.06	1.56E-01	0	1897	0.69	-0.09	0.03	4.86E-04	37	7721
rs17738155	PKHD1	6	51264035	t	c	0.92	-0.24	0.05	5.9E-06	39	5825	0.92	0.06	0.10	5.30E-01	0	1896	0.92	-0.17	0.05	2.51E-04	42	7721
rs4722909	CHN2	7	29481456	a	g	0.60	-0.13	0.03	3.2E-06	0	5823	0.60	0.09	0.05	9.66E-02	40	1894	0.60	-0.08	0.03	9.92E-04	38	7717
rs7922045	FGFR2	10	122991722	t	c	0.26	0.17	0.03	5.7E-07	0	5824	0.23	-0.10	0.06	1.05E-01	35	1824	0.25	0.11	0.03	2.41E-04	39	7648
rs649529	RAB38	11	87647899	t	g	0.43	-0.15	0.03	9.3E-06	0	5825	0.43	-0.12	0.05	1.91E-02	0	1962	0.43	-0.14	0.03	5.84E-07	0	7787

A1 is the coded allele (effect allele), i.e. the beta corresponds to the effect by which UACR changes per each additional copy of the coded allele.  
The I<sup>2</sup> statistic of the combined results was obtained from a separate analysis incorporating each discovery file with single GC-correction and the replication files. Standard error (SE) and p-value of the combined results are based on double-GC corrected results as described in the methods.

**Supplementary Table 9: Association results for the index SNPs near *RAB38/CTSC* and in *HS6ST1* in the DCCT/EDIC Study**

<b>incident microalbuminuria (1244 individuals [268 cases]; primary endpoint)</b>					
SNP	effect allele	frequency of effect allele	effect	se	p-value
rs649529	T	0.42	0.04	0.09	0.64
rs13427836	T	0.14	-0.18	0.14	0.20
<b>time to macroalbuminuria or ESRD (1304 individuals [133 cases]; secondary endpoint)</b>					
SNP	effect allele	frequency of effect allele	effect	se	p-value
rs649529	T	0.42	0.24	0.14	0.09
rs13427836	T	0.14	-0.31	0.22	0.16

Cox proportional hazards regression models were used to estimate hazard ratios after adjustment for cohort status (primary vs. secondary), treatment (intensive vs. conventional), cohort\*treatment interaction (stratified by DCCT year of entry), age of diagnosis squared, sex, diabetes duration squared, body mass index, blood pressure, triglyceride, HDL-C, total cholesterol, smoking (all at baseline), as well as time-dependent updated mean A1C, and time-dependent indicators for hypertension diagnosis and treatment. Imputation quality (rs13427836) and call rate (rs649529) were both  $\geq 0.99$ .